School of Life Sciences

Department of Biological Sciences and Biotechnology

INVESTIGATING THE CONTRIBUTION OF GERMLINE AND SOMATIC VARIANTS IN THE FATTY ACID METABOLISM PATHWAY TO PROSTATE CANCER AGGRESSIVENESS IN SOUTHERN AFRICAN MEN

by

UMUNA MAENDO
18000532
BSc (Biology and Biotechnology)
(Botswana International University of Science and Technology)

A **Thesis** Submitted to the School of Life Sciences in Partial Fulfilment of the Requirements for the Award of the Degree of Master of Science in Biological Sciences and Biotechnology of BIUST

Supervisor(s): Dr. Kebaneilwe Lebani, Dr. Nerve Zhou
Department of Biological Sciences and Biotechnology
School of Life Sciences, BIUST
lebanik@biust.ac.bw, zhoun@biust.ac.bw
Prof. Vanessa Hayes
School of Medical Sciences
University of Sydney
vanessa.hayes@sydney.edu.au

April, 2025

TABLE OF CONTENTS

TABLE OF CONTENTS ..... *i*
LIST OF TABLES ..... *iii*
LIST OF FIGURES ..... *iv*
LIST OF ABBREVIATIONS ..... *v*
DECLARATION REGARDING THE WORK AND COPYRIGHT ..... *vii*
ACKNOWLEDGEMENTS ..... *viii*
ABSTRACT ..... *ix*
CHAPTER 1: INTRODUCTION *1*

1.1 PROBLEM STATEMENT **2**
1.2 GENERAL AIM **2**
1.3 SPECIFIC OBJECTIVES **2**
1.4 HYPOTHESIS **2**
1.5 JUSTIFICATION OF THE STUDY **2**

CHAPTER 2: LITERATURE REVIEW *4*

2.1. PROSTATE CANCER PATHOLOGY **4**
2.2 PROSTATE CANCER EPIDEMIOLOGY **6**
2.3 EPIDEMIOLOGY OF CANCER IN BOTSWANA **8**
2.4 RISK FACTORS **10**

2.4.1 Age 10
2.4.2 Family history 11
2.4.3 Race 12
2.4.4 Genetic alterations 12
2.4.5 Diet 18

2.5 FATTY ACID METABOLISM ASSOCIATION WITH PROSTATE CANCER **19**

2.5.1 Lipid storage and release 21
2.5.2 Fatty acid uptake and transport 22
2.5.3 De novo fatty acid synthesis 22
2.5.4 Fatty acid oxidation 23
2.5.5 Treatment options targeting FAM 24

CHAPTER 3: METHODS *26*

3.1 COMMON VARIANT ANALYSIS **26**

3.1.1 Preliminary methods - exome sequencing 26
3.1.2 Common variant analysis and statistics 27

3.2 RARE GERMLINE AND SOMATIC VARIANT ANALYSIS 28

3.2.1 Preliminary methods - whole genome sequencing 28
3.2.2 Rare germline variant analysis 29
3.2.3 Somatic variant analysis 30

CHAPTER 4: RESULTS 32

4.1 COMMON GERMLINE VARIANT ANALYSIS 32

4.1.1 FAM associated PCa risk alleles 32
4.1.2 FAM risk alleles associated with aggressive PCa 34

4.2 RARE GERMLINE VARIANT ANALYSIS 36

4.2.1 Rare pathogenic variants 37
Table 5: Description of identified rare pathogenic germline variants 37
4.2.2 Rare oncogenic variants 39
4.2.3 Rare variants with predicted loss of function (LoF) 40

4.3 SOMATIC VARIANT ANALYSIS 43

4.3.1 Tumour mutational burden 43
4.3.2 Functional impact prediction 43
4.3.3 Somatic variant landscape 46

CHAPTER 5: DISCUSSION 47
CHAPTER 6: CONCLUSION AND FUTURE WORK 53
REFERENCES 54

LIST OF TABLES

Table 1: Summary of clinical information for the exome study population. 27

Table 2: Summary of clinical information for the patients who underwent WGS. 29

Table 3: Top associated significant variants from a logistic regression of cases (n=451) and controls (n=292). 33

Table 4: Top associated significant variants from a logistic regression of high risk prostate cancer cases (ISUP grade 3-5, n=203) and low risk prostate cancer cases or controls (ISUP grade 1-2/No PCa, n=461). 35

Table 5: Description of identified rare pathogenic germline variants 37

Table 6: Clinical data for PCa patients with rare pathogenic variants. 38

Table 7: Description of identified rare oncogenic germline variants. 39

Table 8: Clinical data for patients with rare oncogenic variants. 39

Table 9: Loss of function variants 40

Table 10: Clinical data for patients with LoF variants 41

Table 11: Potentially damaging variants identified from 53 European-ancestry derived tumours and 105 African-ancestry derived tumours. 44

Table 12: Clinical data of patients with potentially damaging variants. 45

LIST OF FIGURES

Figure 1: Prostate cancer pathology based on Gleason score and ISUP grading. 5

Figure 2. The geographical distribution of prostate cancer in 2022. 6

Figure 3: Age-standardised incidence and mortality rate in the world and in Africa. 7

Figure 4: Description of common and rare germline variants. 13

Figure 5: Algorithm for inherited/germline and tumour/somatic mutation testing in men diagnosed with prostate cancer. 17

Figure 6: Overview of lipid metabolism in prostate cancer. 20

Figure 7: Lipolysis of lipid droplets to release fatty acids 22

Figure 8: Synthesis of fatty acids from citrate in the tumour cell. 23

Figure 9: Overview of fatty acid oxidation. 24

Figure 10: Assessment of rare germline variants for pathogenecity, oncogenecity and loss of function. 30

Figure 11: A Manhattan plot of -log10 p-values from a logistic regression analysis of 451 cases and 292 controls.. 32

Figure 12: A Manhattan plot of 303 variants from 203 HRPCa vs 461 LRPCa. 34

Figure 13. Comparative analysis of rare germline variants for pathogenecity, oncogenecity and loss of function. 36

Figure 14: Box plot of tumour mutational burden. 43

Figure 15: Functional impact prediction using SIFT and PolyPhen. 44

Figure 16: An oncoplot showing the somatic alteration landscape. 46

LIST OF ABBREVIATIONS

ADT	Androgen Deprivation Therapy
ANNOVAR	Annotate Variation
AQPS	Aquaporin
AR	Androgen Receptor
ASIR	Age-Standardized Incidence Rate
ASMR	Age-Standardized Mortality Rate
ATGL	Adipose Triglyceride Lipase
ATP	Adenosine Triphosphate
CACT	Carnitine/Acylcarnitine Translocase
CADD	Combined Annotation Dependent Depletion
CAMP	Cyclic Adenosine Monophosphate
CDKs	Cyclin-Dependent Kinase
CPTs	Carnitine Palmitoyltransferase
CRPC	Castration-Resistant Prostate Cancer
DAG	Diacylglycerol
dbSNP	Single Nucleotide Polymorphisms Database
DGAT1/2	Diacylglycerol Acyltransferase 1/2
ETC	Electron Transport Chain
EWAS	Exome-Wide Association Studies
FA(s)	Fatty Acid(s)
FABP(s)	Fatty Acid-Binding Protein(s)
FAM	Fatty Acid Metabolism
FAO	Fatty Acid Oxidation
FASN	Fatty Acid Synthase
FATP(s)	Fatty Acid Transport Protein(s)
GNOMAD	Genome Aggregation Database
GWAS	Genome-Wide Association Study
HDI	Human Development Index
HRPCA	High Risk Prostate Cancer
HSL	Hormone-Sensitive Lipase
ISUP	International Society Of Urological Pathology
LD(s)	Lipid Droplet(S)
LOF	Loss Of Function
LOFTEE	Loss-Of-Function Transcript Effect Estimator
LRPCA	Low Risk Prostate Cancer
MAF	Minor Allele Frequency
MGL	Monoacylglycerol Lipase
MRI	Magnetic Resonance Imaging
NCCN	National Comprehensive Cancer Network
PCA	Prostate Cancer
PDVs	Potentially Damaging Variants

PKA	Protein Kinase A
POC	Public Oncology Centre
PolyPhen	Polymorphism Phenotyping
PRS	Polygenic Risk Scores
PSA	Prostate-Specific Antigen
PUFAs	Polyunsaturated Fatty Acids
SAPCS	Southern African Prostate Cancer Study
SIFT	Sorting Intolerant From Tolerant
SNP	Single Nucleotide Polymorphism
SNV	Single Nucleotide Variant
SREBPS	Sterol Regulatory Element-Binding Proteins
TAG(S)	Triacylglycerol(S)
TCA	Tricarboxylic Acid
TCGA	The Cancer Genome Atlas
TMB	Tumour Mutational Burden
VCF	Variant Call Format
VEP	Variant Effect Predictor
VUS	Variants of Uncertain Significance
WGS	Whole Genome Sequencing
WHO	World Health Organization

BOTSWANA INTERNATIONAL UNIVERSITY OF SCIENCE & TECHNOLOGY

POSTGRADUATE SCHOOL

Declaration Regarding the Work and Copyright

DECLARATION REGARDING THE WORK AND COPYRIGHT

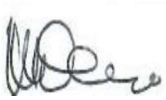
Candidate: UMUNA MAENDO

Student ID: 18000532

Thesis Titled: **INVESTIGATING THE CONTRIBUTION OF GERMLINE AND SOMATIC VARIANTS IN THE FATTY ACID METABOLISM PATHWAY TO PROSTATE CANCER AGGRESSIVENESS IN SOUTHERN AFRICAN MEN**

I, the **Umuna Maendo**, certify that the Thesis is all my own original work and that I have not obtained a degree in this University or elsewhere on the basis of any of this work.

This dissertation/thesis is copyright material protected under the Berne Convention, the Copyright and Neighbouring Rights Act, Act. No. 8 of 2000 and other international and national enactments, in that behalf, on intellectual property. It must not be reproduced by any means, in full or in part, except for short extracts in fair dealing; for researcher private study, critical scholarly review or discourse with an acknowledgement, without the written permission of the office of the Postgraduate School, on behalf of both the author and the BIUST.

Signed:  Date: 23/04/2025

Primary Supervisor: Dr. Kebaneilwe Lebani

I, Dr. Kebaneilwe Lebani, hereby confirm that I have inspected the above titled thesis and to the best of my knowledge, it is based on the original work of the candidate.

Signed:  Date: 23/04/2025

ACKNOWLEDGEMENTS

First and foremost, I would like to express my deepest gratitude to Dr. Kebaneilwe Lebani for her unwavering support, endless patience, and those 3 a.m. emails that reminded me I was never alone in this journey. Your belief in me made all the difference. I am also sincerely grateful to my co-supervisor, Dr. Nerve Zhou, for his continuous encouragement, insightful feedback, and steadfast guidance throughout this project.

A special thank you goes to Professors Riana Bornman and Vanessa Hayes for welcoming me into the world of bioinformatics and genomics with open arms. Thank you for seeing potential in me and giving me the opportunity to grow in this field. To the amazing team at the Hayes Lab; Weerachai, Pam, Jenna, Jue, and Kazzem, thank you for your kindness and patience when I had a million questions. I have learned so much from each of you, both professionally and personally. I am deeply grateful to the patients and their families, whose data made this research possible. Thank you for your contributions, your courage, and your trust. I also acknowledge all the dedicated urologists and pathologists involved in the collection and curation of this valuable data; your work underpins everything I have done.

To my Mom and Dad, your endless encouragement and eternal support have carried me through every challenge. To my wonderful aunties, Becky and Jandjee, thank you for instilling in me a love for learning and a passion for education. And to my sisters and friends, your laughter, love, and unwavering support gave me strength and joy in moments when I needed it most.

ABSTRACT

Prostate cancer (PCa) is a significant global health issue, ranking second in cancer diagnoses and is the fifth leading cause of cancer-related deaths among men worldwide. Men of African ancestry have disproportionately higher PCa-associated mortality rates compared to other racial groups, underscoring a critical health disparity that necessitates further research. Despite this, most PCa genome studies focus predominantly on populations of European ancestry, excluding African men from tailored diagnosis and treatment options. PCa progression is facilitated by altering various signalling and metabolic pathways including fatty acid metabolism (FAM). To support their rapid growth, PCa cells meet their higher energy demands primarily through the oxidative degradation of fatty acids. While genes involved in FAM have been extensively studied, it is not well understood how both germline and somatic mutations within FAM influence patient predisposition and PCa progression. This project sought to address this gap by investigating the impact of germline and somatic variants within the FAM pathway on PCa aggressiveness in men of African and European ancestry. Data generated from the Southern African Prostate Cancer Study (SAPCS) was used in this study. A total of 743 Black South African men participating in the SAPCS underwent whole-exome genotyping. Common variants (MAF > 0.01) in 158 FAM genes were tested for association with PCa (451 cases vs 292 controls) and PCa aggressiveness (203 high risk, ISUP 3-5, vs 461 low risk, ISUP 1-2 and controls) using logistic regression. The significant variants were assessed for deleterious or damaging effects using SIFT and PolyPhen. Rare germline and somatic variant analysis were performed on whole-genome sequencing data from 158 treatment-naive individuals with PCa (Australia: n=53; South Africa: n=105). Rare variants (MAF < 0.05) were annotated with ANNOVAR, ClinVar, InterVar, and cancer genome interpreter as well as functional impact prediction tools (SIFT, PolyPhen, CADD, LOFTEE). The somatic variants were assessed for deleteriousness using SIFT and PolyPhen and tumour mutational burden (TMB) of the somatic variants was calculated. Nine common SNPs in FAM-related genes were significantly associated with PCa, including three deleterious variants in *D2HGDH, EHHADH* and *CA2*. Eight SNPs were linked to aggressive PCa, with deleterious variants in *INMT* and *AOC3*. These common SNPs had not been previously associated with PCa. Rare germline analysis revealed 15 pathogenic variants, four oncogenic variants and 15 variants with predicted loss of function. 80% of individuals with these rare variants had aggressive PCa. Somatic analysis showed African tumours had a higher (though not statistically significant) mean TMB than European tumours (p-value 0.056). Several PDVs were exclusive to African-derived tumours, including mutations in *FASN, ACADL, ACOT8*, and *ADH1C*. Although limited by a small sample size, this study reveals novel germline and somatic variants in FAM pathway genes, highlighting their contribution to PCa risk and aggressiveness. Future research should prioritise African population inclusion, increased sample size, and include phenotypic studies in PCa genomics to improve diagnostics, treatment, and health equity.

CHAPTER 1: INTRODUCTION

Prostate Cancer (PCa), as defined by the World Health Organization, is a malignancy originating within the prostate gland, a small, walnut-shaped organ that plays a crucial role in the male reproductive system. PCa is among the most commonly diagnosed malignancies in men globally and is a leading cause of cancer-related mortality, particularly in older males. It presents a complex and heterogeneous disease spectrum, ranging from indolent tumours to highly aggressive and metastatic forms. While widespread implementation of prostate-specific antigen (PSA) testing and improvements in imaging and therapeutic strategies have contributed to earlier detection and improved survival rates, prostate cancer still imposes a substantial health burden, especially in men of African descent.

PCa is one of the most heritable cancers in the world however significant gaps remain in understanding the molecular and genetic factors that drive prostate cancer onset, progression, and treatment response. A major limitation of current genomic research is its overwhelming exclusion of African-ancestry derived data [1]. Large-scale projects such as The Cancer Genome Atlas (TCGA) have yielded critical insights into prostate cancer genomics, however, their underrepresentation of non-European populations has limited the generalizability of findings [2]. This lack of diversity is particularly problematic given the clear evidence that men of African descent are disproportionately affected by prostate cancer, exhibiting higher mortality rates, earlier onset, and more aggressive clinical features.

Emerging research has highlighted lipid metabolism as a central hallmark of cancer, with deregulated lipid biosynthesis and uptake supporting the energetic and structural demands of rapidly proliferating tumour cells [3, 4]. In prostate cancer specifically, the involvement of lipid metabolic processes is especially noteworthy. This is because the prostate gland naturally synthesizes and secretes large quantities of lipids, including citrate and polyunsaturated fatty acids, for seminal fluid production [5]. Tumorigenesis in the prostate co-opts these processes, leading to enhanced lipid synthesis and accumulation that support malignant transformation, proliferation, and therapy resistance.

Alterations in FAM genes can influence tumour behaviour, modulate immune responses, and alter sensitivity to treatment [4, 6]. However, despite their biological significance, the contribution of inherited and somatic genetic variation in FAM-related genes to prostate cancer aetiology remains poorly understood, particularly in African populations. This study aims to bridge these knowledge gaps by conducting a comparative genomic analysis of prostate cancer samples from South African (African ancestry) and Australian cohorts (European ancestry). By focusing on genes involved in fatty acid metabolism, this work seeks to uncover population-specific mutations that may underlie differences in disease risk between these two populations. Through this approach, the study aspires to enhance the global understanding of prostate cancer and contribute toward more equitable precision medicine strategies.

1.1 PROBLEM STATEMENT

Despite growing evidence of ancestry-specific genomic differences in prostate cancer, African populations remain underrepresented in genomic studies, limiting our understanding of functionally relevant variants that may influence prostate cancer progression and therapeutic outcomes. One particularly understudied area in PCa research is the mutational processes involved in fatty acid metabolism (FAM), which has been increasingly implicated in prostate cancer tumorigenesis and resistance to therapy. To the best of our knowledge, no study to date has specifically investigated the contribution of FAM-associated variants to PCa risk and adverse outcomes in African men.

1.2 GENERAL AIM

To determine the contribution of genetic variants in the fatty acid metabolism pathway towards driving PCa aggressiveness in men of Southern African ancestry.

1.3 SPECIFIC OBJECTIVES

- To assess the contribution of rare germline variants in the FAM pathway towards predisposing Southern African men to aggressive PCa.
- To assess the contribution of common germline pathogenic variants in the fatty acid metabolism pathway towards predisposing Southern African men to aggressive PCa.
- To determine the contribution of somatic variants in the fatty acid metabolism pathway towards predisposing Southern African men to aggressive PCa.

1.4 HYPOTHESIS

Men of Southern African ancestry will exhibit significant, deleterious alterations in the FAM pathway, characterized by an elevated tumour mutational burden, and a greater number of predicted deleterious mutations. These specific genomic changes contribute to the higher aggressiveness and poorer clinical outcomes observed in men of Southern African ancestry.

1.5 JUSTIFICATION OF THE STUDY

Addressing disparities in prostate cancer research, particularly the lack of genomic data from African populations, is crucial given the high burden of the disease in this demographic. Specific mutations in fatty acid metabolism (FAM) genes have not been explicitly detailed in African populations, making this study a novel investigation into the mutational landscape of FAM genes across men of African ancestry and men of European ancestry. This research will provide new insights into ancestry-specific variants and their functional implications in disease progression.

Such insights are crucial for improving biomarker discovery, tailoring therapeutic strategies, and advancing precision oncology in Southern African populations.

CHAPTER 2: LITERATURE REVIEW

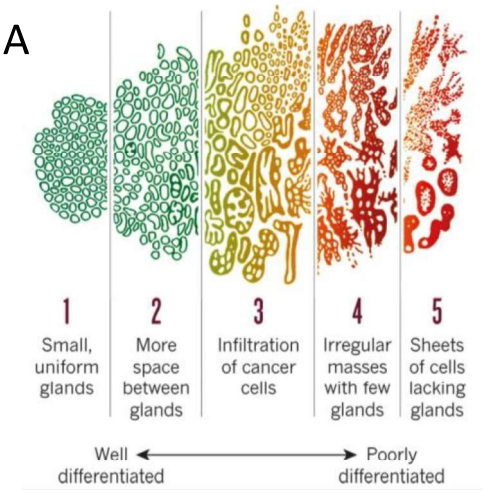
2.1. PROSTATE CANCER PATHOLOGY

Prostate cancer (PCa) can be classified into various histological types, with acinar adenocarcinoma being the most prevalent [7]. This type originates in the glandular cells of the prostate, typically grows slowly, and is often detected early. Prostatic intraepithelial neoplasia (PIN)-like adenocarcinoma, once considered a distinct entity, has now been reclassified in the 2022 WHO Classification of Urinary and Male Genital Tumours (5^{th} edition) as a variant of acinar adenocarcinoma due to similar prognosis and outcomes [8]. Ductal adenocarcinoma, which develops in the ducts of the prostate, is less common but more aggressive, with a greater tendency to spread beyond the prostate. Transitional cell carcinoma, arising from the urethra or bladder and invading the prostate, is rare and highly aggressive. Small cell carcinoma and other neuroendocrine tumours are extremely rare but exhibit aggressive behaviour, often presenting with advanced disease and poor prognosis. Another development is the formal recognition of treatment-related neuroendocrine prostate cancer (t-NEPC) as a distinct tumour type [8]. This aggressive form of cancer often emerges following androgen deprivation therapy or novel androgen receptor-targeted therapies, representing a mechanism of treatment resistance.

PCa diagnosis involves several clinical and laboratory techniques aimed at detecting the disease at an early and treatable stage. One of the most commonly used methods is the PSA test. PSA is a glycoprotein produced by the epithelial cells of the prostate and is normally found in semen but can also circulate in the bloodstream [9]. During PSA testing, a blood sample is analysed, and levels above 4 ng/mL typically warrant further investigation. However, PSA is not cancer-specific and can be elevated in benign conditions such as benign prostatic hyperplasia [9, 10]. As a result, elevated PSA levels alone are insufficient for a definitive diagnosis. To complement PSA testing, clinicians may perform a Digital Rectal Examination (DRE), which involves palpating the prostate through the rectal wall to detect abnormalities such as lumps or hard areas [11]. Magnetic resonance imaging (MRI), particularly multiparametric MRI, has emerged as an important imaging modality to localize suspicious lesions and guide targeted biopsies [12].

When abnormalities are detected through PSA, DRE, or MRI, a prostate biopsy is often performed to confirm the presence of cancer. This procedure involves using a thin, hollow needle to collect prostate gland tissue samples, which are then examined under a microscope by a pathologist [13]. The histological analysis of biopsy samples allows for tumour grading using the Gleason scoring system. Pathologists evaluate the cancer cells and assign a grade from 1 to 5 based on their appearance under the microscope [14]. They then identify the two most predominant patterns and add their respective grades to produce the Gleason score (figure 1A). For improved clarity and standardization, the International Society of Urological Pathology (ISUP) developed a grading system that classifies tumours into five grade groups, with higher groups indicating more aggressive disease and poorer prognosis (Figure 1B) [15].

A



B

Gleason Score	ISUP Grade Group
6 (3+3)	1
7 (3+4)	2
7 (4+3)	3
8 (4+4, 3+5, 5+3)	4
9 or 10 (4+5, 5+4, 5+5)	5

Low risk

High risk

Figure 1: Prostate cancer pathology based on Gleason score and ISUP grading [16]. **A**. Gleason score is calculated based on the two most common patterns of cancer cell arrangement. **B**. The ISUP grade groups are based on the Gleason scores, with the higher the grade group, the more the risk of the tumour becoming more aggressive. ISUP-International Society of Urological Pathologists.

As prostate cancer advances, it can begin to manifest through various symptoms that prompt medical evaluation. One of the earliest and most common signs is trouble urinating, which can include difficulty initiating urination, a weak or interrupted urine stream, or increased frequency of urination. These urinary symptoms are often the result of tumour growth exerting pressure on the urethra or surrounding structures. Some individuals may notice blood in their semen, a condition known as hematospermia, or experience discomfort and pain in the pelvic area [17]. Advanced stages of the disease are often associated with metastasis, particularly to the bones of the spine, pelvis, and hips, leading to persistent, deep bone pain. Erectile dysfunction can also arise as the tumour impacts nerves and blood vessels related to sexual function [18].

Treatment options for prostate cancer vary depending on factors such as PSA level, tumour grade and stage, the risk of recurrence, and patient preference [13, 19]. In cases of low-risk or localized prostate cancer, active surveillance may be considered. This strategy involves regular monitoring without immediate treatment, delaying interventions until signs of progression appear [12]. For patients requiring active treatment, options include surgery, radiation therapy, hormonal therapy, chemotherapy, and cryotherapy. Radical prostatectomy, the surgical removal of the prostate and surrounding tissues, is often employed in localized disease and may be used alongside radiation therapy [20]. Chemotherapy, particularly with agents like docetaxel, is used in advanced stages but can come with substantial side effects [21]. Cryotherapy, which involves freezing prostate tissue to kill cancer cells, is another minimally invasive option for selected cases [13]. Despite the effectiveness of these treatments, each carries the risk of adverse effects such as fatigue, hair loss, urinary incontinence, sexual dysfunction, and haematological complications [13]. Resistance to treatment can also develop over time, emphasizing the need for personalized and adaptive therapeutic approaches [22, 23].

2.2 PROSTATE CANCER EPIDEMIOLOGY

Prostate cancer represents a significant worldwide health issue, ranking second only to lung cancer in terms of diagnosis and standing as the fifth leading cause of cancer-related deaths among men [24]. Globally it is the eighth leading cause of cancer death and the fourth most diagnosed cancer [25]. The severity of PCa is emphasized by the staggering statistics from 2022, which documented approximately 1 467 854 new cases and 397 430 deaths attributed to PCa [26]. The global burden of PCa is predicted to continue to escalate, with an estimated 2.3 million new cases and 740,000 deaths by 2040. Notably, PCa is the most commonly diagnosed cancer in 112 countries and the leading cause of cancer-related mortality in 48 countries [26].

The epidemiological landscape of PCa exhibits significant geographical disparities (figure 2). The age-standardized incidence rates (ASIRs) in Northern America and Oceania are significantly higher, more than 70 cases per 100 000 males, than in Africa and Asia, below 31 cases per 100,000 males [27]. Although Africa has the second lowest ASIRs, the continent has the highest age standardized mortality rates (ASMRs), 17.3 per 100 000 males, followed by Latin America and the Caribbean (LAC), 13.3 per 100 000 males, an indication of the association between African ancestry and PCa risk [28]. Asia is the least affected continent, with the lowest ASIRs and the lowest ASMRs [27]. The Southern African region is particularly affected by PCa as it has the highest incidence and mortality rates in the African region (figure 3).

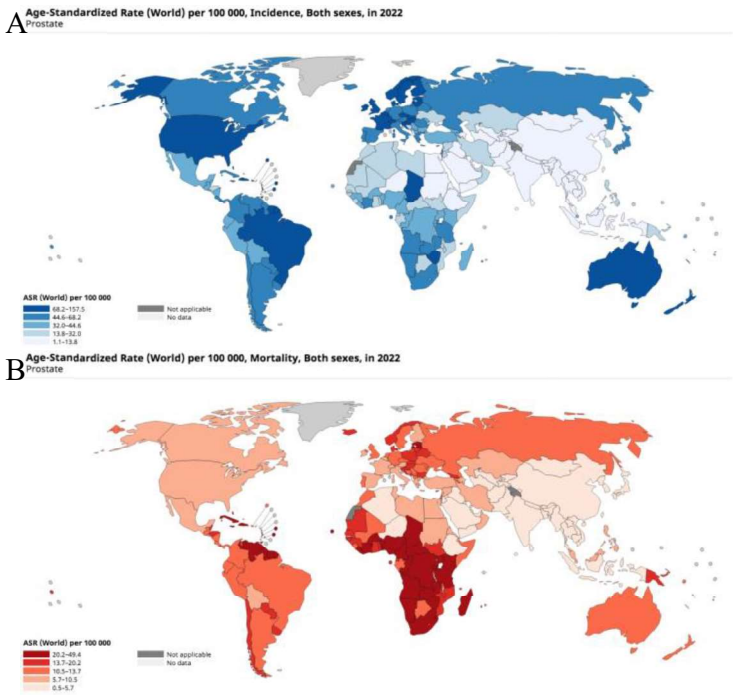


Figure 2. The geographical distribution of prostate cancer in 2022 [29]. A- Age-standardised incidence rates, B- Age-standardised mortality rates. Incidence is negatively correlated with mortality in Sub-Saharan Africa.

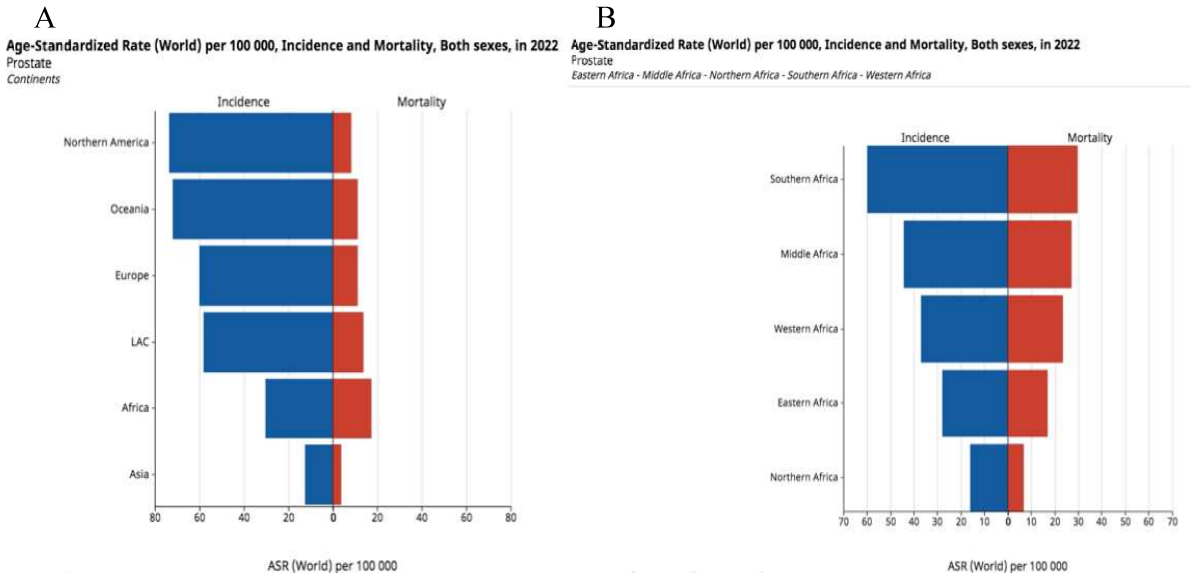


Figure 3: Age-standardised incidence (blue) and mortality rate (red) in the world (A) and in Africa (B) [29]. Southern Africa carries the highest burden of prostate cancer in both incidence and mortality in Africa.

Several studies have proven that the human development index (HDI), a consolidated measure of the average achievement across key dimensions of human development within each country including life expectancy, income per capita and education index, influences PCa incidence and mortality [27, 28, 30, 31]. Countries with very high HDI, such as USA, UK and Australia, exhibit ASIRs that are more than double those with low HDI [27]. This has partly been attributed to widespread PSA screening. A study from 2018 indicated that the incidence rates of PCa increased by 51% in the span of eighteen years following regularized PCa screening [20]. Notably, the United States accounted for more than a fifth of the incidence cases in 2017, validating the association between high HDI and elevated ASIRs. On the contrary, several Asian countries lacking organized screening programs, including China, Thailand, Georgia, and Korea, have also experienced rising incidence rates [27]. This has been linked to increased obesity, for example, an increased prevalence of obesity of more than 20% was observed in the past 20 decades. Although ASIRs have been positively correlated with HDI, even in countries with high HDI such as USA, men of African descent are more at risk.

Unlike ASIRs, the burden of ASMRs is negatively correlated with HDI, also a result of systemic PCa screening and developed treatment procedures [32]. Despite the rising incidence, advancements in diagnostic and treatment modalities have contributed to declining mortality rates, even in Africa and Asia, with the highest Estimated Annual Percentage Change (EAPC) observed in high HDI countries (Mahal et al., 2022; Wang et al., 2022). PSA screening, coupled with active surveillance and improved therapies, has played a pivotal role in reducing PCa mortality. The Göteborg screening trial demonstrated a 35% decrease in mortality rate in a period of 18 years, highlighting the interplay between socioeconomic factors and disease burden (Wang et al., 2022).

2.3 EPIDEMIOLOGY OF CANCER IN BOTSWANA

According to *GLOBOCAN 2022*, prostate cancer is the fourth most diagnosed cancer in Botswana (6.9%) and is the number one cancer in men (16.9%) [33]. In terms of mortality, PCa accounts for 6% of all cancer related deaths, ranking fifth in Botswana, while it is responsible for 13.8% of cancer-related deaths in men, placing it third after Kaposi's sarcoma and oesophageal cancer [34]. Cancer mortality in Botswana is alarmingly high, with approximately 75% of patients presenting with advanced-stage disease [35]. This highlights the need for early detection and improved cancer care services.

Botswana's Ministry of Health and Wellness, in collaboration with the World Health Organization (WHO), has formulated a national strategy targeting four major non-communicable diseases namely; cancer, diabetes, cardiovascular diseases, and chronic respiratory diseases. This initiative aims to mitigate cancer incidence by addressing modifiable risk factors such as smoking and physical inactivity [36]. The strategy, however, does not have any guidelines on PCa management, screening, diagnosis and treatment. PCa screening remains uncommon in the public healthcare sector. Limited screening programs contribute to late-stage diagnosis, delayed treatment initiation, and poor patient survival rates, particularly for prostate cancer [37]. The Botswana government has also established four hospitals across different regions as designated public oncology centres (POCs). Three POCs conduct PSA testing and two perform DRE, however none perform ultrasound-guided prostate biopsies. These procedures are only available in private hospitals at government expense in Gaborone and Francistown [35].

Prostatectomy, surgical removal of the prostate, is not available in Botswana, resulting in a heavy reliance on radiation therapy as the primary treatment for prostate cancer. When prostatectomy is unavailable, reliance on radiation therapy alone may limit treatment options and impact patient outcomes. While radiation can shrink or destroy cancer cells, it may not provide the same long-term control as surgery for localized prostate cancer, potentially increasing the risk of recurrence or metastasis [38]. Additionally, without surgery, early detection of recurrence through PSA monitoring may be delayed. Radiation therapy may also lead to long-term side effects, affecting patients' quality of life, and for advanced prostate cancer, it may not be sufficient on its own, often requiring a combination of treatments like surgery or hormonal therapy for optimal disease control [39].

Until recently, radiation therapy was only available at Gaborone Private Hospital. However, in 2023, a public radiotherapy facility was established at Sir Ketumile Masire Teaching Hospital in Gaborone, significantly enhancing accessibility for patients in need of cancer treatment[40]. Other recent advances include large-scale initiatives such as Potlako+ Study, a community-randomized trial led by the Botswana Harvard AIDS Institute Partnership (BHP) to improve timely cancer detection and treatment initiation [41]. Another initiative from BHP is the Thabatse Cancer Observational Cohort, a study analyzing cancer types, stages, treatments, and outcomes among patients receiving cancer care in Botswana [42].

Despite its prevalence amongst men, prostate cancer is amongst the most understudied cancers in Botswana [43]. A study published in 2025 assessing alpha-methyl acyl-CoA racemase (AMACR/p504s) and p63 as diagnostic markers demonstrated their high sensitivity and specificity in distinguishing prostate cancer from benign prostatic hyperplasia [44]. This study analyzed 96 archived, formalin-fixed paraffin-embedded FFPE prostate tissue specimens collected between 2013 and 2019 from the only two public health anatomical pathology laboratories in Botswana: The National Health Laboratory in Gaborone and Nyangabgwe Referral Hospital in Francistown prostate samples. To date, this is the only study to utilize prostate samples from Botswana in research. Despite these advancements, Botswana still has a long way to go in establishing comprehensive national prostate cancer guidelines, expanding screening programs, and improving access to diagnostic and treatment options to enhance patient outcomes and reduce the burden of the disease.

2.4 RISK FACTORS

Prostate cancer is associated with various risk factors [30, 45]. Obesity, low fitness levels, diabetes, certain dietary patterns, and even vitamin E supplementation have emerged as potential risk factors [44]. The precise mechanisms behind these associations remain unclear, but factors such as increased consumption of energy, animal fats, and red meats may contribute to the heightened risk of aggressive PCa [47]. Lifestyle factors such as smoking also affect PCa outcomes, with studies indicating higher overall mortality and recurrence rates among smokers [45, 48, 49]. Notably, smokers exhibit a higher frequency of certain somatic genetic mutations, including those in genes like *PREX2, KMT2C, AGO2*, and *PTEN*, which may contribute to poorer clinical outcomes [50]. The major risk factors for PCa are age, family history of PCa or breast cancer, African ancestry, and genetic alteration and diet has also been implicated in PCa aggressiveness.

2.4.1 Age

Age is a well-established risk factor for PCa, with increasing incidence and mortality rates observed in older populations [51]. The lifetime probability of PCa diagnosis is estimated at 12.5%, with a sharp rise from 1.8% in men aged 50–59 years to 9.0% in men 70 years and older [52]. Autopsy studies have further revealed that approximately 40% of unscreened men over 60 years harbour undiagnosed PCa, a figure that increases to 60% in men over 80 years [51]. Notably, 32% of these cases are classified as ISUP grade group ≥2, indicating clinically significant disease.

Prostate cancer can be broadly classified into early-onset (diagnosed before age 55) and late-onset (diagnosed after age 55) [53]. Early-onset PCa tends to be more aggressive and is often linked to genetic predispositions, including germline mutations in *BRCA1/2* and *HOXB13* genes . Younger patients typically present with higher Gleason scores and ISUP grade groups, indicating a greater likelihood of clinically significant disease [14, 54, 55]. Conversely, late-onset PCa is more frequently detected due to screening and is often indolent, though aggressive forms are still observed in older populations [56].

A risk-adapted screening strategy, typically commencing at 50 years of age, is often advised by medical organisations such as the European association of urology or the national comprehensive cancer network (NCCN) [11]. However, for high-risk populations, such as men of African descent and individuals with a family history of PCa, screening initiation at 40 years is recommended. With the increasing risk of clinically significant PCa with age, determining the optimal age for initiating and discontinuing screening remains a critical debate. Many guidelines recommend against PSA screening in men over 70–75 years due to concerns about overdiagnosis and limited evidence of mortality reduction [57, 58] . However, some studies argue that a uniform cessation of PSA screening at 70 years may be premature, advocating for a more individualized approach to screening cessation and follow-up during active surveillance [59, 60].

Age also significantly influences treatment decisions for PCa [11]. Younger men diagnosed with clinically significant disease are more likely to undergo radical treatment options such as radical prostatectomy or radiation therapy due to their longer life expectancy and potential for disease progression [61] . Conversely, older men, particularly those with comorbidities, may be managed with active surveillance or watchful waiting to minimize the risks associated with overtreatment.

Additionally, older men are more likely to experience lower overall survival rates despite treatment [23]. Hormone therapy is commonly employed in elderly patients with advanced disease to slow progression, while chemotherapy is typically reserved for those with metastatic or castration-resistant prostate cancer (CRPC) [62].

2.4.2 Family history

Prostate cancer has the highest heritability rates among cancers, meaning that genetic factors significantly contribute to its development. Twin studies estimate that heredity accounts for approximately 57% of PCa cases [63]. The likelihood of developing PCa increases with the number of affected relatives, with individuals having a first-degree relative diagnosed with PCa facing a 2.5-fold higher lifetime risk [64]. Additionally, aggressive forms of PCa tend to cluster within certain families, suggesting a link between genetic predisposition and disease severity [64, 65]. Some research suggests that familial PCa is more likely to recur and result in poorer outcomes after radical prostatectomy (RP) [53].

A Swedish study found that survival rates were similar among fathers and sons diagnosed with PCa, further supporting its hereditary nature [64]. The Health Professionals Follow-Up Study, which monitored 3,695 PCa patients between 1986 and 2004, found that individuals with both a father and brother diagnosed with PCa had a 2.3-fold increased risk (95% CI = 1.76–3.12). A father-only or brother-only diagnosis resulted in a 2.16-fold and 1.95-fold increased risks respectively [53]. Interestingly, a 2023 study examining PCa-related mortality reported a lower mortality rate among individuals with a strong family history of the disease, possibly due to increased awareness and early detection efforts [66].

Families with a history of PCa have been observed to have higher occurrences of other cancers, particularly breast cancer [67]. Despite developing in different organs, multiple studies suggest a genetic connection between these two cancers [68–70]. A study by Ren et al., (2019) found that individuals with a first-degree relative diagnosed with female breast cancer had a 21% higher risk of developing PCa and a 34% increased risk of lethal disease [71]. Additionally, a family history of PCa alone was associated with a 68% higher risk of overall disease and a 72% greater risk of lethal disease. Individuals with a family history of both breast and prostate cancer exhibited an even greater risk of lethal PCa, though statistical significance varied across studies [66, 68–70].

Age has also been identified as a modifier of the relationship between family history and PCa risk. Among men under 60, those with a family history of breast cancer faced a 62% increased risk of developing PCa, while a family history of PCa was linked to a 2.19-fold increased risk [72]. Additionally, having a family history of both breast and prostate cancer was associated with a 2.44-fold increased risk of PCa in men under 60. It is therefore crucial to perform early screening and genetic counselling for individuals with a significant family history of cancer.

2.4.3 Race

Prostate cancer incidence and mortality exhibit significant disparities across racial and ethnic groups. Afro-Caribbean and sub-Saharan African men experience some of the highest mortality rates from PCa (figure 2). The rates for prostate cancer mortality are substantially lower in other racial groups, such as Asian/Pacific Islanders, and Europeans. This indicates that PCa mortality may be related to African ancestry. Studies have shown that African American men have a notably higher rate of prostate cancer than their Caucasian and Asian counterparts [73]. This disparity is further evidenced by the fact that African American men are more likely to be diagnosed with prostate cancer at an earlier age and often with more advanced or metastatic stages of the disease, leading to poorer outcomes when compared to other racial groups [74, 75]. This is further compounded by higher PSA levels at diagnosis and an increased risk of mortality compared to Caucasian patients [76].

A review by McHugh et al. (2021) proposed that the higher burden of prostate cancer in African American men could be traced back to the genetic makeup of West and West-Central African populations, from which many African Americans are descended [77]. These populations have been shown to carry genetic variants that increase susceptibility to prostate cancer, contributing to the disease's early onset and more aggressive presentation [78, 79]. However, even within African populations, differences in prostate cancer incidence and outcomes exist due to large genetic diversity. In African men, the highest rates of prostate cancer are found in men of Southern African ancestry (figure 3), which is often attributed to the genetic predispositions in these populations. Furthermore, studies show that Khoisan ancestry in Black South African patients is linked to a significantly higher risk of prostate cancer compared to Bantu ancestry, underscoring the complex genetic factors at play within African groups [80]. These genetic differences, combined with environmental and socio-economic factors, including underrepresentation in clinical trials and genetic research, hamper the development of targeted therapies for this population, explain why men of African descent are more affected [1, 2]. With increasing recognition of the genetic underpinnings of prostate cancer, particularly in African descent populations, there is a growing need for inclusive research and tailored treatment strategies to address these disparities in prostate cancer outcomes.

2.4.4 Genetic alterations

2.4.4.1 Common germline variants

About 57% of variation in prostate cancer is attributed to genetic factors [81]. Increased PCa risk can be due to rare germline variants, defined as a minor allele frequency (MAF) <1%, as well as the combined effects of common variants, typically at MAF >5% but typically also including intermediate risk (MAF 1-5%) variants [82]. The ‘Common Disease, Common Variant’ theory suggests that although common variants individually have less effect on disease risk, collectively they cause common diseases such as PCa [83] (Figure 4). The rationale is that variants with a large effect on disease risk would likely reduce reproductive fitness and thus become rare over

generations. Since common diseases are prevalent, the variants causing them, likely do not affect reproductive fitness and persist across generations.

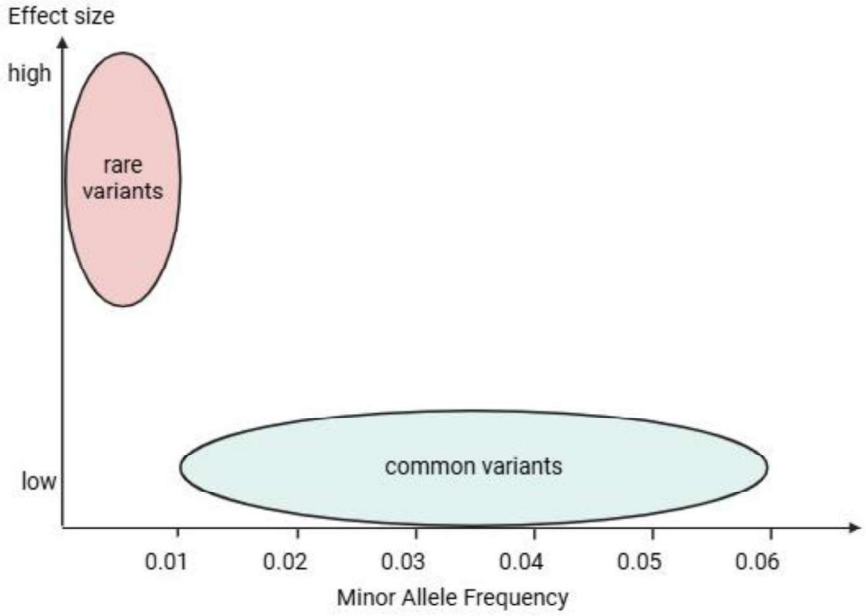


Figure 4: Description of common and rare variants. Common variants (MAF > 0.05) are more prevalent in a population and have less effect on disease expression than rare variants which are less prevalent (MAF < 0.01) but are more likely to contribute to disease expression when present.

The completion of the Human Genome Project and advancements in both Genome-Wide Association Studies (GWAS) and Exome Wide Association Studies (EWAS) have significantly advanced research on genetic susceptibility to common diseases, including PCa. Genome-wide association and fine-mapping studies of PCa have identified germline risk variants. Unfortunately, these studies were primarily conducted in European populations and although recent studies are advocating for African inclusion, African American individuals whose ancestry is mostly West African are included and the Southern African population remains underrepresented [84–86]. Common germline variants are used to develop polygenic risk scores (PRS) which predict genetic risk for developing PCa and other common diseases, potentially improving targeted screening and early detection [84, 85]. PRS are aggregate measures of the effects of common variants on PCa risk. A study by Conti et al., (2021), showed that men of African ancestry had 2.18 times higher PRS than men of European ancestry [89]. Consequently, studying the contribution of common germline variants in African men, who have a higher incidence of aggressive PCa, could inform improved risk prediction models and guide more personalized screening and treatment approaches [88].

2.4.4.2 Rare germline variants

Rare pathogenic germline variants have been linked to both early-onset and aggressive forms of PCa. Notable examples include rare pathogenic variants in *BRCA2* and *HOXB13.* Both genes have been shown to have a substantial impact on both PCa risk and disease aggressiveness [90]. The *HOXB13* G84E missense variant has been linked to early-onset and familial PCa, with a greater effect observed in aggressive cases [91]. Men with metastatic PCa are more likely to carry pathogenic variants in DNA-repair genes *BRCA2, ATM,* and *CHEK2* than those with localized disease [92], while overall, a higher prevalence of protein-truncating variants have been observed in men with metastatic as opposed to indolent PCa [93].

As the frequency of genetic mutations differs across ethnicities, contributing to disparities in PCa outcomes, it is critical that there be population representative data. This has been highlighted by recent studies advocating for the inclusion of African genomes in the identification of rare germline variants associated with PCa [94–97]. A study by Wei et al., (2023) discovered two rare variants that were significantly associated with PCa risk in African American men, p.R14Q in *GPRC5C* and p.R511Q in *IGF1R*. Another study by Darst et al., (2022) demonstrated that a rare germline variant in the *HOXB13* gene (rs77179853), was found exclusively in men of West African descent, and was linked to a higher risk of both overall and advanced PCa [99]. Additionally, when using data from African American populations, a loss-of-function variant in the DNA repair gene *ADPRHL1* (c.A233T; p.D78V) triggers prostate tumorigenesis and increases susceptibility to PCa [78], while *HOXB13* (X285K) is associated with a higher risk of more aggressive PCa, particularly at a younger age [79].

Rare germline variants associated with PCa risk have been used to develop germline testing panels. The purpose of these germline testing gene panels is prognosis, advising treatment and to screen for men at high-risk or very high-risk for PCa, defined as ISUP grade group 4 or 5 and/or PSA ≥ 20ng/ml, by NCCN (version 4.2023) [100]. Germline testing can reveal mutations classified as pathogenic or likely pathogenic, which may expand prostate cancer treatment possibilities, provide access to clinical trials, and highlight the risk of other cancers [101]. Additionally, a positive result suggests a 50% likelihood that immediate family members carry the same genetic risk. As a result, patients are encouraged to share their test findings with relatives and consider referring them to genetic counselling for further evaluation through cascade genetic testing [102].

Current PCa germline testing panels are focused on only 20 genes, namely *RAD50, RAD51C, RAD51D, ATM, CHEK2, PALB2, TP53, PMS2, MUTYH, BRCA1, BRCA2APC, EPCAM, HOXB13, BRIP, FANCA, MLH1, MSH2, APC, MSH6* and *NBN*. Despite African ancestry being a risk factor for aggressive PCa, many of the rare germline studies are conducted in primarily European populations [94]. Indeed, Gheybi et al, (2023) showed that current germline testing criteria exclude for men of African ancestry as these 20 genes were shown to be under represented by pathogenic variants in Southern African men, and there was a high number of novel oncogenic and pathogenic variants [94].

While common genetic variants identified through GWAS have provided valuable insights into PCa susceptibility, the role of rare germline pathogenic variants in predicting aggressive disease

is becoming increasingly clear, with much needed focus on men of African ancestry. The integration of germline testing into clinical practice holds promise for improving the precision of PCa risk assessment and guiding personalized treatment strategies, ultimately enhancing outcomes for men at risk of aggressive PCa.

2.4.4.3 Somatic variants

Somatic variants are DNA alterations that arise after conception, occurring in any cell except germ cells, and are not inherited [103]. The majority of PCa cases are considered sporadic, meaning genetic damage happens randomly after birth, primarily driven by somatic mutations [103, 104]. However, while most somatic mutations found in tumours have no significant impact on cancer progression, referred to as passenger mutations, a small fraction (<0.1%) are classified as driver mutations [103]. The most common, known somatic alteration in PCa is the fusion of androgen-dependent transmembrane protease serine 2 (*TMPRSS2*) and erythroblast transformation-specific-related gene (*ERG*) [105]. This fusion is more prevalent in men of European descent (49%) than in men of African descent (25%) and presumably occurs in early tumorigenesis. Certain somatic mutations arising during tumour progression can induce alterations in non-tumour cells within the tumour microenvironment, to enable the tumour to escape detection by the immune system [106]. For example, somatic mutations in *PTEN, TP53, RB1*, and *CDK12* may facilitate immune evasion [106]. Interestingly, African American men are significantly more likely to harbour pathogenic variants in *CDK12* than Caucasian Americans [107, 108]. Patients with *CDK12*-altered PCa exhibit an aggressive tumour and present with poor outcomes to hormonal, taxane and poly ADP-ribose polymerase (PARP) inhibitors [109].

The most mutated genes in localized PCa according to a study by Fraser and Rouette, (2019) are *TP53, FOXA1,* and *SPOP* [1]. Driver mutations in *TP53* and *FOXA1* are enriched in metastatic CRPC, while somatic SNVs in *SPOP* are more common in localized PCa and confer sensitivity to CYP17A1 inhibitors like abiraterone [110]. Metastatic CRPC accumulates 2-3 times more non-synonymous SNVs than localized disease due to additional mutations and clonal selection from long-term Androgen Deprivation Therapy (ADT). Additionally, somatic variants in DNA damage repair genes occur in ~19% of metastatic PCa [111]. Other significant genes include *KIT, KRAS,* and *MED12* mutations which are linked to aggressive disease and tumorigenesis [104, 112].

Due to the limitations of PSA testing in reliably diagnosing and monitoring prostate cancer (PCa), there is growing interest in identifying novel biomarkers for prognosis and diagnosis. One such avenue involves the detection of tumour-acquired variants. However, sequencing cancer genomes poses challenges compared to germline sequencing because cancer evolves through mutations over time, leading to genomic heterogeneity and sub-clonal variations [101, 103, 113]. Additionally, contamination with non-malignant cells in solid tumours reduces the detection of tumour-specific mutations [101]. Despite these challenges, several FDA-approved somatic tests are available. These include assays for homologous recombination repair gene mutations and markers of

microsatellite instability and mismatch repair deficiency, which can have direct therapeutic implications [114]. Additionally, some next-generation sequencing based somatic tests may report investigational alterations such as Androgen Receptor (AR) amplifications, *PTEN* deletions, PI3K/Akt/mTOR pathway mutations, and *TMPRSS2-ERG* gene fusions that could inform eligibility for clinical trials or emerging targeted therapies [115].

The NCCN guidelines (version 4.2023) recommend somatic testing via next-generation sequencing for patients with regionally advanced or metastatic prostate cancer (Figure 5) in order to inform treatment options[116]. For example, patients with human homologous repair mutations are advised to use PARP inhibitors for treatment [117]. The interplay between rare inherited genetic variations and somatic alterations can affect cancer's clinical outcome. Germline risk alleles found in gene regulatory elements, may influence specific genes leading to somatic alterations. For example, research by has revealed an interaction between PCa risk alleles and the somatic loss of *PTEN* or *RB1* [118].

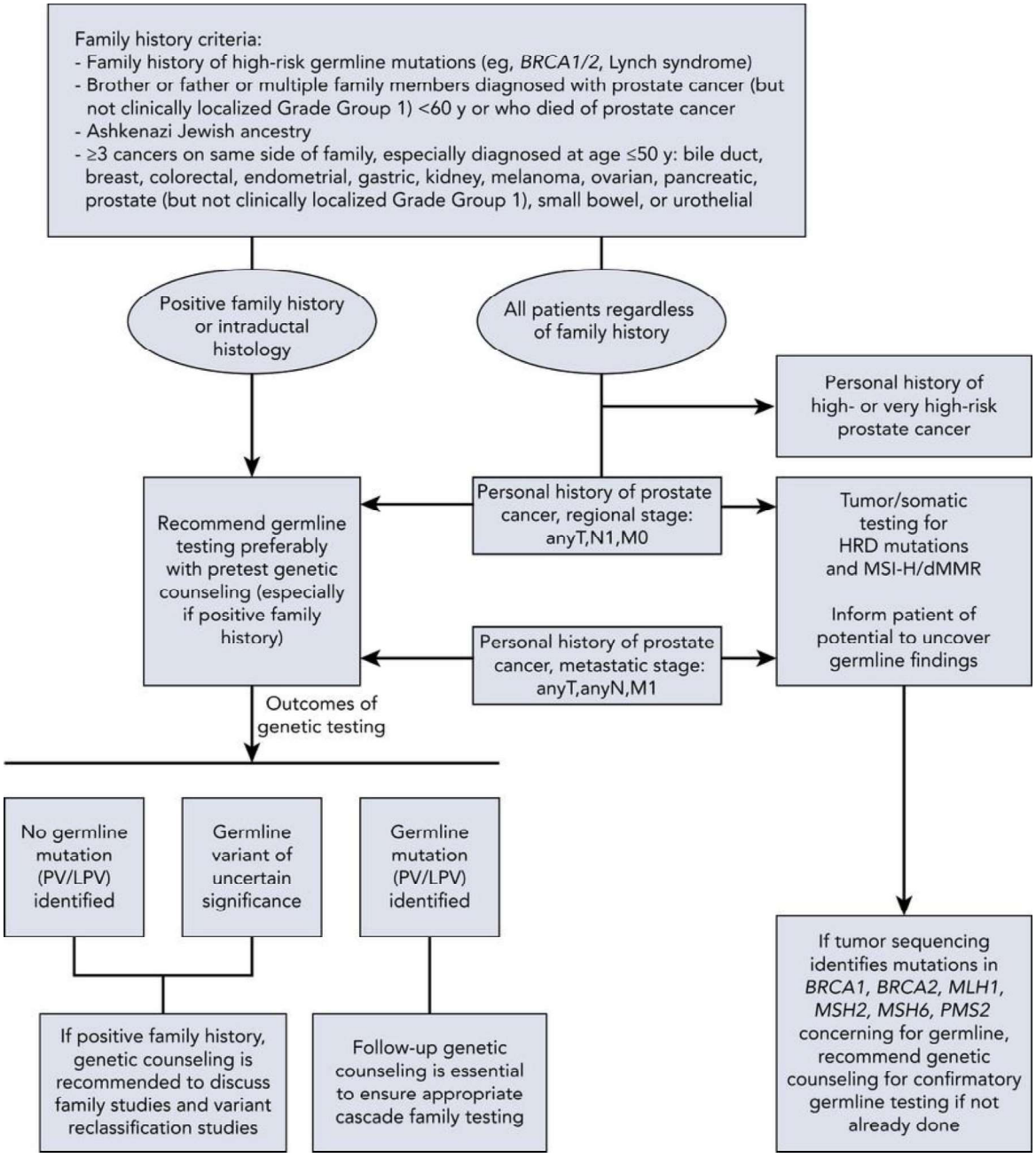


Figure 5: Algorithm for inherited/germline and tumour/somatic mutation testing in men diagnosed with prostate cancer [102]. dMMR - mismatch repair deficiency; HRD - homologous recombination DNA repair; MSI-H - microsatellite instability-high; PV/LPV - pathologic variant or likely pathologic variant.

2.4.5 Diet

Dietary intake directly influences fatty acid profiles. Diets rich in omega-3 polyunsaturated fatty acids (PUFAs) found in fatty fish, flaxseeds, and walnuts are associated with a lower risk of prostate cancer, likely due to their anti-inflammatory properties that help suppress tumour development and progression [119]. Conversely, diets high in omega-6 PUFAs (commonly derived from vegetable oils) and with a high omega-6 to omega-3 ratio have been linked to increased risk, as they can promote a pro-inflammatory environment favourable to cancer initiation [120]. Moreover, high consumption of saturated fats, prevalent in red and processed meats, is correlated with increased PCa risk (Aucoin et al., 2017). Saturated fats can trigger inflammation and oxidative stress, contributing to tumorigenesis. Additionally, a high-fat diet can elevate circulating bioactive lipids, which stimulate the proliferation and migration of cancer cells, particularly those with low lipolytic activity [122]. In obese individuals, lipid-rich tumour microenvironments driven by activated adipocytes and elevated systemic lipid levels further support tumour growth and malignancy [123].

Prostate tumours often exhibit distinct alterations in fatty acid composition compared to adjacent normal tissue. These changes are especially pronounced in more aggressive forms of the disease, with tumours showing increased levels of saturated fatty acids (SFAs) and reduced levels of PUFAs, particularly omega-3 PUFAs. [120]

2.5 FATTY ACID METABOLISM ASSOCIATION WITH PROSTATE CANCER

Prostate cancer exhibits a unique metabolic profile that relies heavily on lipid metabolism. As tumours develop, crosstalk between adipose tissue and cancer cells leads to changes in adipocyte function and paracrine signalling, promoting a microenvironment that supports tumour growth [123]. Over the past decade, it has become evident that tumour cells co-opt adipocytes in the tumour microenvironment, converting them into cancer-associated adipocytes [124]. This interaction results in metabolic symbiosis, where adipocytes and cancer cells exchange metabolites through bidirectional signalling that shifts cancer cell metabolism from glycolysis to lipid-dependent energy production. PCa is particularly influenced by its proximity to peri-prostatic adipose tissue, which secretes the chemokine CCL7. This chemokine migrates to the peri-prostatic region and promotes tumour cell invasion through its receptor CCR3. [125]

Unlike other cancers, prostate adenocarcinoma remains highly dependent on AR signalling [126, 127]. ARs play a critical role in regulating fatty acid synthesis and oxidation to meet the bioenergetic and anabolic demands of PCa cells. Additionally, AR influences lipid uptake, storage, cholesterol metabolism, and phospholipid synthesis. ADT is the most effective systemic treatment for PCa, achieving a 90% response rate by inhibiting androgen synthesis from both the testes and adrenal glands. However, when PCa recurs as CRPC, it continues to rely on AR signalling despite low serum androgen levels. This is achieved through AR overexpression, amplification, mutation, production of ligand-independent AR variants, and AR cistrome reprogramming [128]. ARs enhance both the expression and activation of transcription factors called sterol regulatory element-binding proteins (SREBPs), directly regulating the transcription of lipogenic genes due to the proximity of AR binding sites to lipid metabolism gene promoters (Figure 6)[129]. Interestingly, SREBPs can reciprocally activate AR gene expression, further amplifying lipid metabolism in PCa.

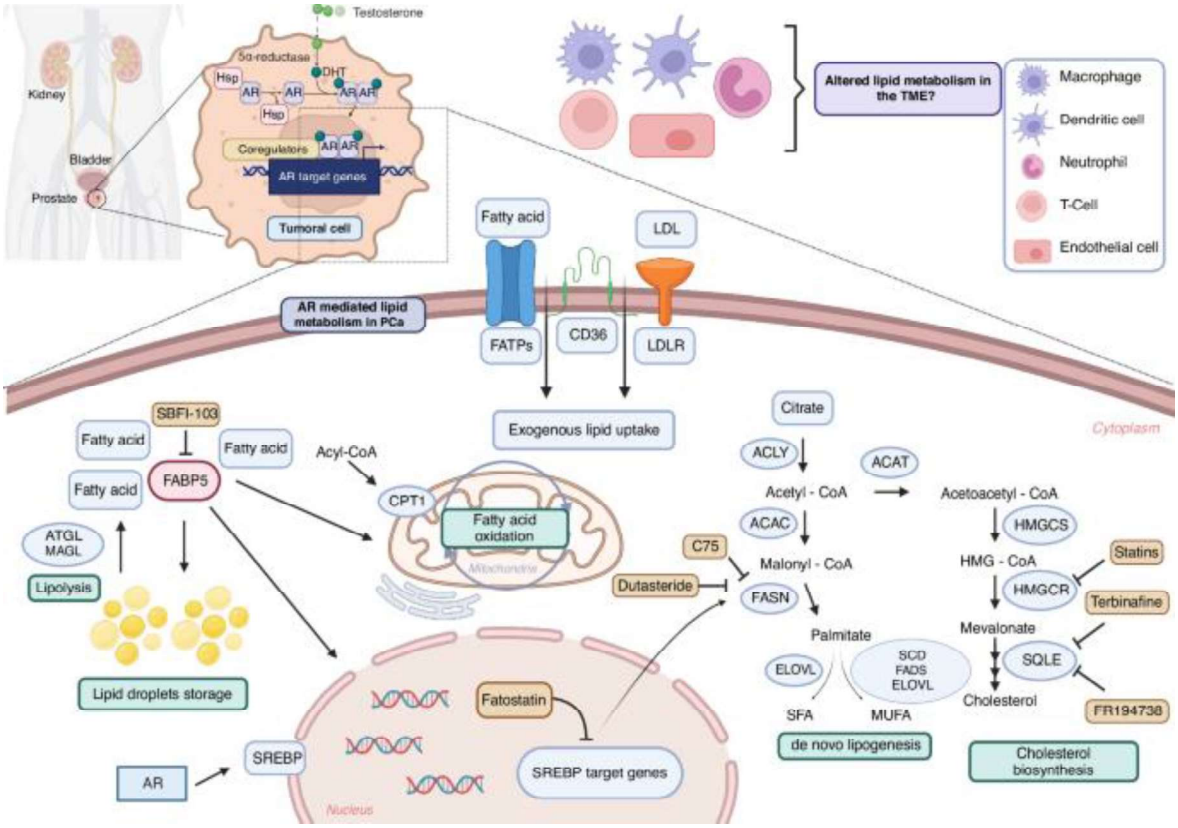


Figure 6: Overview of lipid metabolism in prostate cancer [130]. AR signalling activates SREBPs, transcription factors that regulate expression of various genes involved in FAM such as CPT1, FASN and ELOVL. Fatty acids (FAs) are derived from either lipolysis of lipid droplets within the tumour cell or from FA transporters such as CD36. The FAs are then metabolised for energy generation or for signalling molecules that can alter the tumour micro environment by suppressing immune response. AR – androgen receptor, LDL – low density lipoprotein, TME – tumour microenvironment, SREBPs – sterol regulatory element binding proteins.

Beyond AR-dependent mechanisms, PCa-associated genetic aberrations also contribute to metabolic reprogramming. PI3K mutations, p53 loss, and *PTEN* deletion enhance lipid metabolism through oncogenic signalling pathways [1]. Similarly, the PI3K/AKT/mTORC1 pathway plays a crucial role in SREBP activation to promote synthesis of monounsaturated fatty acids that promote resistance to cancer cell death via ferroptosis [133]. The co-deletion of *PTEN* and promyelocytic leukemia protein, and the myelocytomatosis oncogene results in SREBP-dependent lipogenesis, further promoting PCa metastasis [134].

Besides energy generation, FAM is also important for the migration of cancer cells to promote metastasis and epithelial to mesenchymal transition, facilitated by fluid membranes composed of cholesterol and saturated fatty acids [4]. In addition, fatty acids and their derivatives, eicosanoids, act as secondary messengers in signalling pathways that promote tumorigenesis, cell proliferation and immunosuppression [34]. FAM is also crucial in sustaining cancer cells during metabolic stress. For example, NADH is essential in the detoxification of reactive oxygen species and cells can also synthesize saturated phospholipids which combat peroxidation induced by reactive

oxygen species [119]. As prostate cancer cells become more reliant on fatty acids, they often divert glucose metabolism towards the pentose phosphate pathway, which provides them with the necessary nucleotides and NADPH for rapid growth and proliferation. This metabolic shift aids in the synthesis of DNA and RNA, facilitating cell division [28]. PCa exhibits an active lipophagy mechanism and numerous abnormalities in lipid metabolism, including increased; lipid uptake from circulation, de novo synthesis of fatty acids and phospholipids, transfer of fatty acids from stromal adipocytes into PCa cells, fatty acid oxidation and phospholipid content.

2.5.1 Lipid storage and release

Circulating fatty acids (FAs) primarily originate from triglyceride breakdown in lipoproteins or adipose tissue lipolysis [135]. Within cells, lipid droplets (LDs) function as key organelles for storing neutral lipids like triglycerides (TAGs) and sterol esters, encased within a phospholipid monolayer [131]. The accumulation of LDs is a metabolic hallmark of PCa, offering protection against lipotoxicity [136]. The predominant pathway for TAG synthesis in most tissues is the glycerol-3-phosphate pathway. Glycerol-3-phosphate acyltransferases initiate TAG synthesis by converting glycerol-3-phosphate and fatty acyl-CoA into lysophosphatidic acid (LPA). Acylglycerolphosphate acyltransferases subsequently catalyse the conversion of LPA into phosphatidic acid (PA). Phosphatidic acid phosphatases then transform PA into diacylglycerol (DAG), which serves as a precursor for the final step of TAG formation [131]. This crucial last step is facilitated by diacylglycerol acyltransferases (DGAT1 and DGAT2), which esterify DAG with an additional fatty acyl-CoA to produce TAGs. Notably, DGAT1 is significantly overexpressed in PCa [137]. The TAGs are then deposited between the lipid bilayer membrane of the endoplasmic reticulum, inducing the formation of LDs by the proteins seipin and perilipin3 [138].

Lipolysis mobilizes stored lipids for energy production. This process is initiated by adipose triglyceride lipase (ATGL), followed by hormone-sensitive lipase (HSL) and monoacylglycerol lipase (MGL) to convert TAG to diacylglycerols then into glycerol and fatty acids [139–142] (Figure 7). While ATGL's role in PCa remains unclear due to conflicting reports, HSL is known to contribute to CRPC by promoting intra-tumoural steroid synthesis that activates AR signalling [143]. MGL is highly expressed in aggressive PCa cells, and is linked to epithelial-to-mesenchymal transition and tumour progression [144]. Notably, MGL inhibition reduces PCa aggressiveness, highlighting its potential as a therapeutic target [145]. An alternative lipolysis pathway involves protein kinase A (PKA) activation, which phosphorylates HSL and perilipin, a key lipid droplet-associated protein [146]. This phosphorylation facilitates HSL docking to LDs and triggers lipolysis. Prolonged PKA activation results in LD fragmentation, increasing surface area for lipase action. Lipolysis releases FAs and glycerol, fuelling energy production through FA oxidation (FAO) and enhancing the metabolic flexibility of PCa cells.

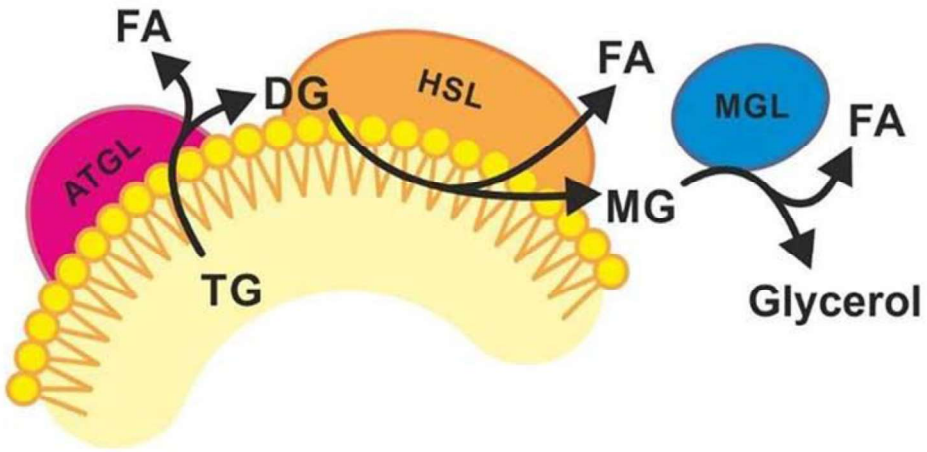


Figure 7: Lipolysis of lipid droplets (yellow) to release fatty acids [140]. This process involves the subsequent release of FAs during the breakdown of TG into DG then MG by enzymes ATGL, HSL, MGL. FA - fatty acids, ATGL - adipose triglyceride lipase, HSL - hormone sensitive lipase, MGL - monoacylglycerol lipase, DG - diacylglyceride, MG – monoacylglycerol

2.5.2 Fatty acid uptake and transport

Fatty acids (FAs) enter cells through receptor families such as fatty acid transport proteins (FATPs), fatty acid-binding proteins (FABPs), and fatty acid translocase CD36 - a multifunctional glycoprotein receptor [147, 148]. FATPs, part of the SLC27A family, consist of six members with tissue-specific expression patterns that facilitate the uptake of long-chain FAs [131]. FABPs, located in the plasma membrane, actively transports FAs like palmitate and oleate into cells, while cytosolic FABPs regulate intracellular FA trafficking, influencing storage, oxidation, and membrane incorporation [149]. Among these receptors, CD36 is particularly relevant in cancer metabolism. Highly expressed in PCa, CD36 enhances lipid uptake, supporting tumour growth, angiogenesis, and metastasis [150]. Its elevated expression correlates with poor prognosis across various cancers, reinforcing its role in tumour progression [151, 152]. The upregulation of FA transport proteins in PCa increases lipid availability, promoting metabolic adaptation and tumour survival.

2.5.3 De novo fatty acid synthesis

The process of endogenous FA synthesis begins in the cytosol with the conversion of citrate, derived from the mitochondrial tricarboxylic acid (TCA) cycle, into acetyl-CoA by ATP citrate lyase (ACLY). Acetyl-CoA is subsequently converted to malonyl-CoA by the rate-limiting enzyme acetyl-CoA carboxylase (ACACA, also known as ACC). FASN then catalyses the formation of palmitate, a 16-carbon saturated fatty acid (SFA) [153]. Following its synthesis, palmitate undergoes further modifications. Fatty acyl-CoA desaturases and stearoyl-CoA desaturases introduce double bonds, generating monounsaturated fatty acids (MUFAs), while

elongases (ELOVLs) extend the carbon chain, producing PUFAs [154] (Figure 8). These newly synthesized fatty acids can be incorporated into phospholipids for membrane biosynthesis or stored as triglycerides. The dysregulation of SREBP activity, particularly due to increased MAPK pathway activation, has been linked to an enhanced abundance of intracellular saturated and monounsaturated fatty acyl chains, promoting metastatic progression in PCa models [155]. This suggests that de novo FA synthesis is regulated by SREBPs.

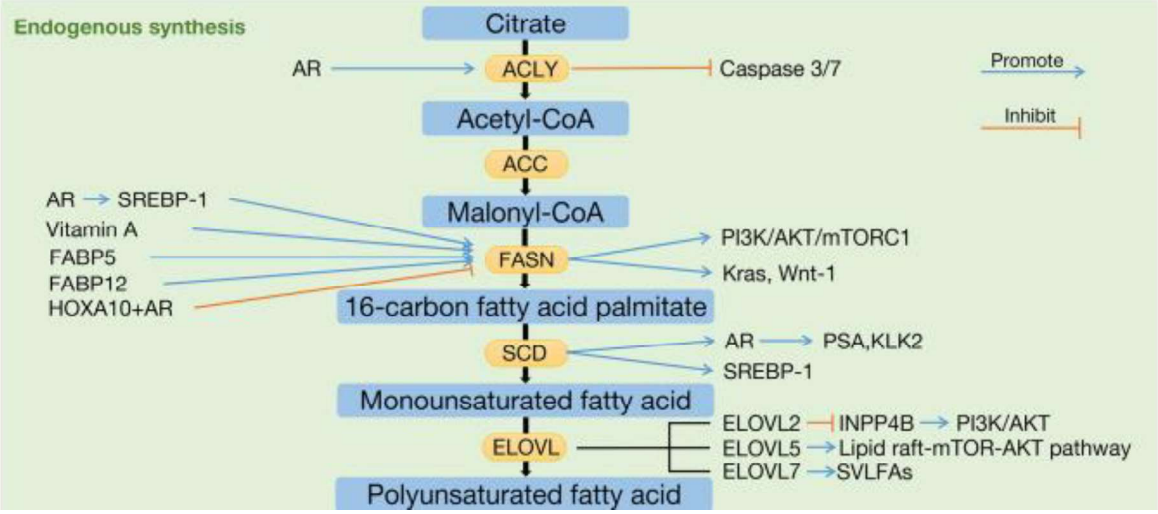


Figure 8: Synthesis of fatty acids from citrate in the tumour cell [125]. SVLFAs – saturated very long chain fatty acids, AR – androgen receptors, SREBPs – sterol regulatory element binding proteins.

2.5.4 Fatty acid oxidation

Fatty acid oxidation (FAO) is a complex, multi-step pathway that occurs in both mitochondria and peroxisomes. In mitochondria, long-chain fatty acids undergo β-oxidation. In contrast, peroxisomes initiate the oxidation of very long-chain fatty acids and branched-chain fatty acids before transferring them to mitochondria for further processing [131]. A critical step in mitochondrial FAO is the transport of long chain fatty acids, facilitated by the carnitine shuttle system, which consists of carnitine palmitoyltransferase 1 (CPT1), carnitine/acylcarnitine translocase (CACT), and carnitine palmitoyltransferase 2 (CPT2). CPT1 has three isoforms, CPT1A, CPT1B, and CPT1C, that work together to catalyze the conversion of fatty acyl-CoA into fatty acyl-carnitine, enabling its transport across the mitochondrial membranes [128]. CACT then moves acyl-carnitine into the mitochondrial matrix, where CPT2 regenerates fatty acyl-CoA, allowing β-oxidation to proceed [131]. Metabolomic and transcriptomic analyses reveal elevated CPT1 and CPT2 expression in PCa, emphasizing their roles in tumour metabolism and survival [156, 157].

Inside the mitochondria, FAO proceeds through a four-step enzymatic process involving acyl-CoA dehydrogenase, enoyl-CoA hydratase, hydroxyacyl-CoA dehydrogenase, and 3-ketoacyl-CoA thiolase (3-KAT). This cycle progressively shortens fatty acid chains by removing two-carbon units as acetyl-CoA, which subsequently enters the (tricarboxylic acid) TCA cycle and undergoes

complete oxidation to CO_2. FAO, in conjunction with the TCA cycle, generates reducing equivalents (NADH and FADH2) that drive ATP production via the electron transport chain (ETC) (Figure 9) [158]. Recent studies indicate that FAO supports the development of castration resistance in PCa [159, 160]. The enzyme 2,4-dienoyl-CoA reductase 1 (DECR1), which facilitates PUFAs β-oxidation by converting 2,4-dienoyl-CoA thioesters into 3-trans-enoyl-CoA, is crucial for mitochondrial lipid degradation [161]. Inhibiting DECR1 disrupts FAO and impairs CRPC progression, underscoring its therapeutic potential [162].

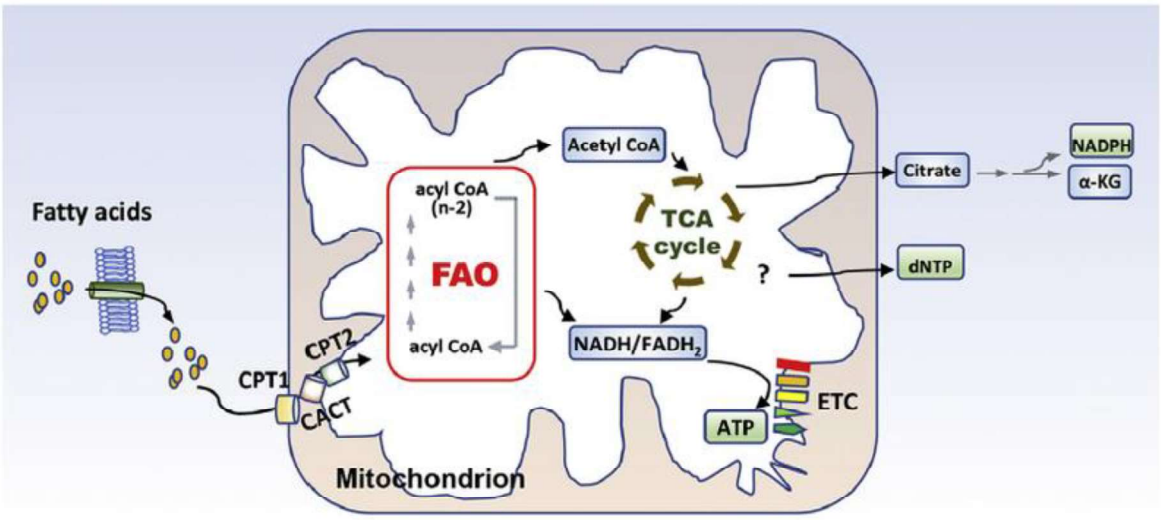


Figure 9: Overview of fatty acid oxidation [147]. Fatty acids from enter mitochondrion through CPTs and are oxidised by successive removal of 2-carbon units (acetyl CoA) which enter the TCA cycle for energy generation. FAO – fatty acid oxidation, ATP – adenosine triphosphate, ETC – electron transport chain, TCA – tricarboxylic acid, α-KG – alpha-ketoglutarate

2.5.5 Treatment options targeting FAM

Prostate cancer is highly dependent on lipid metabolism, making it a promising target for therapeutic interventions. Various strategies have been developed to inhibit fatty acid synthesis, uptake, and metabolism to impede tumour growth and enhance the efficacy of standard treatments. One approach involves targeting fatty acid uptake and transport. CD36 - a key receptor facilitating fatty acid uptake - is implicated in tumour growth and metastasis [150]. Neutralizing antibodies targeting *CD36* such as FA6.152 and JC63.1 selectively block fatty acid uptake, showing therapeutic potential in preclinical models [163, 164]. Additionally, *SBFI-103*, a competitive inhibitor of *FABP5*, has demonstrated efficacy in both in vitro and in vivo PCa models resistant to ADT or taxanes [165]. Combining *FABP5* inhibitors with chemotherapeutic agents like docetaxel or cabazitaxel has also shown positive synergistic effects [166].

Another strategy focuses on inhibiting de novo lipogenesis. *FASN* inhibitors such as TVB-2640, C75, and dutasteride target *FASN*, a crucial enzyme in lipid biosynthesis [167]. Dutasteride indirectly reduces *FASN* expression by inhibiting 5α-reductase, which is responsible for converting testosterone to dihydrotestesterone, preventing binding and activation of Ars [168]. C75 on the

other hand competitively inhibits *FASN* to prevent fatty acid synthesis [169]. Additionally, inhibiting SREBPs by fatostatin, an inhibitor of SREBP cleavage-activating protein (SCAP), prevents SREBP activation and reduces lipid biosynthesis, leading to tumour suppression [170]. Furthermore, inhibition of fatty acid elongase *ELOVL5* using transient RNA interference, has demonstrated anti-tumour effects and may counteract resistance to enzalutamide, an AR inhibitor [171].

Activation of AMP-activated protein kinase (AMPK) has emerged as a metabolic stress-inducing strategy in PCa treatment [172]. AMPK negatively regulates lipogenesis under energy stress, and its activation by drugs such as AMPK activator 5-aminoimidazole-4-carboxamide-1-β-D-ribofuranoside (AICAR), rosiglitazone, and MT 63-78 inhibits fatty acid synthesis and PCa cell proliferation [173–175]. Targeting fatty acid metabolism presents a promising strategy for PCa treatment. While several inhibitors have shown efficacy in preclinical models, further clinical validation is required to establish their role in standard therapeutic regimens.

CHAPTER 3: METHODS

This project uses genomic data generated by the University of Pretoria Faculty of Health Sciences in collaboration with the Hayes Lab from the University of Sydney. All ethical considerations were approved by University of Pretoria Faculty of Health Sciences Research Ethics Committee (HREC #43/2010) with US Federal wide assurance (FWA00002567 and IRB00002235 IORG0001762). Genomic interrogation was approved by St. Vincent's Sydney HREC (#SVH/15/227). Although data is shared between the University of Pretoria and the University of Sydney, it remains the property of the University of Pretoria.

3.1 COMMON VARIANT ANALYSIS

3.1.1 Preliminary methods - exome sequencing

Participants were recruited at the time of PCa diagnosis from Southern African Prostate Cancer Study (SAPCS) urology clinics in the Gauteng and Limpopo Provinces of South Africa. The study included men who self-identified as Black South Africans recruited between 2010 and 2015. The study population underwent whole exome genotyping using the Infinium HumanExome-12 v1.0 BeadChip array and genotypes were called using the Illumina GenomeStudio v2.0 software [176]. As shown in Table 1, from the 798 study population, 451 confirmed cases and 292 controls (N=743) with or without benign prostate hyperplasia remained after quality control and filtering by Soh et al., (2023) [176]. The variants called by Soh et al., (2023) were then used in this study for further analyses.9

Table 1: Summary of clinical information for the exome study population. Of the 451 cases, 203 were identified as high-risk (ISUP 3-5) and 169 as low-risk (ISUP 1-2). Including the 292 controls in the low-risk group, the final comparison included 203 high-risk individuals versus 461 low-risk individuals, with 79 cases lacking an ISUP grade.

Age	Cases (N=451)	Controls (N=292)
Mean	70.52	69.99
SD	9.21	9.07
Median	71	70
Range	49 – 102	45 – 99
PSA (ng/mL)	Counts (%)	Counts (%)
≤ 4	5 (1.15%)	59 (20.85%)
4.01 - 9.99	23 (5.28%)	81 (28.62%)
10 - 19.99	55 (12.61%)	80 (28.27%)
20 - 49.99	80 (18.35%)	43 (15.19%)
50 - 99.99	77 (17.66%)	10 (3.53%)
□ 100	196 (44.95%)	10 (3.53%)
Missing	37	24
ISUP Grade	Counts (%)	
1	122 (32.8%)	
2	47 (12.63%)	
3	46 (12.37%)	
4	80 (21.51%)	
5	77 (20.7%)	
Missing	79	

Abbreviations: PSA, prostate-specific antigen; ISUP, International Society of Urological Pathology.

3.1.2 Common variant analysis and statistics

BiomaRt on R (Ensembl v111) [177] was used to extract the genomic positions for each of the 158 genes [178] in the FAM pathway according to Liberzon et al., (2015), then variants that are within these genes were extracted using Plink v1.9 [179]. The resulting variant call format (VCF) files were used in this study. Performing classical case-control analysis for 305 SNPs from 451 PCa cases *vs* 292 controls, the study had a power of 0.949 to detect associations with a prevalence of 0.001 [176], and a significance threshold of 0.05. Furthermore, 303 SNPs were assessed for aggressiveness using criteria defined as 203 high risk PCa (ISUP 3-5) vs 461 low risk (ISUP 1-2 and controls) with a power of 0.928. From the 743 participants, those without an ISUP grade were excluded. Study power was calculated using the Genetic Association Study Power Calculator [180]

Logistic regression analysis was performed using Plink v1.9, after which the output was used to generate a Manhattan plot using ggplot2 [181] in R to visualize the distribution of the SNPs across the genome as well as the significance of the SNPs. A threshold of 1.5 was used to assess the significance of the SNPs. The MAF in both cases and controls, along with age-adjusted odds ratios (OR) and P-values, were computed using Plink v1.9. For top significant SNPs, the inverse odds ratio was calculated to accurately represent the odds of the risk allele in cases relative to controls. Functionality prediction tools namely SIFT[182] and PolyPhen [183], from SNPnexus available at https://www.snp-nexus.org/v4/ , were used to assess for deleteriousness and the damaging potential of associated SNPs, respectively. The software tools ClinVar[9] available at https://www.ncbi.nlm.nih.gov/clinvar/ and InterVar[185] available at https://wintervar.wglab.org/ were then used for clinical interpretation to identify pathogenic variants.

3.2 RARE GERMLINE AND SOMATIC VARIANT ANALYSIS

3.2.1 Preliminary methods - whole genome sequencing

The study involved 158 treatment-naive individuals with PCa recruited between 2016 and 2021 from Australia (n = 53), and South Africa (n = 105). DNA was extracted from fresh tissue (used for somatic variant analysis) and matched blood samples (used for germline variant analysis), followed by whole-genome sequencing (WGS) using the Illumina NovaSeq platform. A summary of the cohort characteristics is shown in Table 2. The sequencing reads were aligned to the human reference genome (hg38 + alternative contigs) using Burrows-Wheeler Aligner (Sequence Alignment Tool) (v.0.7.15) [186]. Further analysis and filtering was performed according to Jaratlerdsiri et al., (2022). Variants called by Jaratlerdsiri et al., (2022) were used in this study for downstream analysis as shown in figure 10. BiomaRt on R (Ensembl version 111) [177] was again used to extract the genomic positions for each of the 158 genes [178] in the FAM pathway and the somatic and germline variants within those genes were extracted.

Table 2: Summary of clinical information for the patients who underwent WGS. The cohort included 53 Australians and 105 South Africans.

Ancestry	European	African
Patients, N	53	105
Mean [SD] age	61.2 [6.2]	67 [8.5]
Mean [SD] PSA	9.4 [5.2]	254.4 [667.7]
ISUP grade		
1	7 (13.2%)	11 (10.5%)
2	0.0	6(5.7%)
3	0.0	10(9.5%)
4	9(17.0%)	37(35.2%)
5	37(69.8%)	36(34.2%)
Unknown	0.0	5 (4.8%)

Abbreviations: PSA – prostate specific antigen, SD – standard deviation

3.2.2 Rare germline variant analysis

The variants were initially filtered based on minor allele frequency (<0.05) using Plink 1.9 package [179] (Figure 10). Next, the software tool ANNNOVAR [188] was used to annotate the rare variants. Variants that were benign or likely benign in ClinVar and InterVar were removed. Variants were classified as potentially damaging variants (PDVs) by functional impact prediction tools SIFT [172] and PolyPhen [183]. Variants with a SIFT score of ≤ 0.5 or identified by PolyPhen as "Possibly Damaging" or "Probably Damaging" were kept for further analysis. Variants that were a stop-gain, missense or splice donor, as well as those with a combined annotation dependent depletion (CADD) [189] score of ≥ 20 - the higher the score, the greater the deleteriousness - were also retained as PDVs. PDVs were further assessed for pathogenicity using the National Centre for Biotechnology Information's (NCBI) ClinVar database [190] and the InterVar tool that is based on guidelines from the American College of Medical Genetics and Genomics (ACMG) and the Association for Molecular Pathology (AMP)(Li & Wang, 2017). Variants that were 'pathogenic', 'likely pathogenic', or 'affects' were considered pathogenic variants. The Cancer Genome Interpreter web-based server [191] was used to assess for PDVs with oncogenic potential. The oncogenic drivers were modelled in pymol to observe any structural changes to the proteins. Additionally, the loss-of-function transcript effect estimator (LOFTEE) plugin of variant effect predictor (VEP) was used to identify variants that had high confidence loss of function (LoF) [192]. The minor allele frequencies (MAF) of candidate variants identified in the study were compared to those reported in the external gnomAD database (v4.1)to assess population relevance and frequency consistency [193].

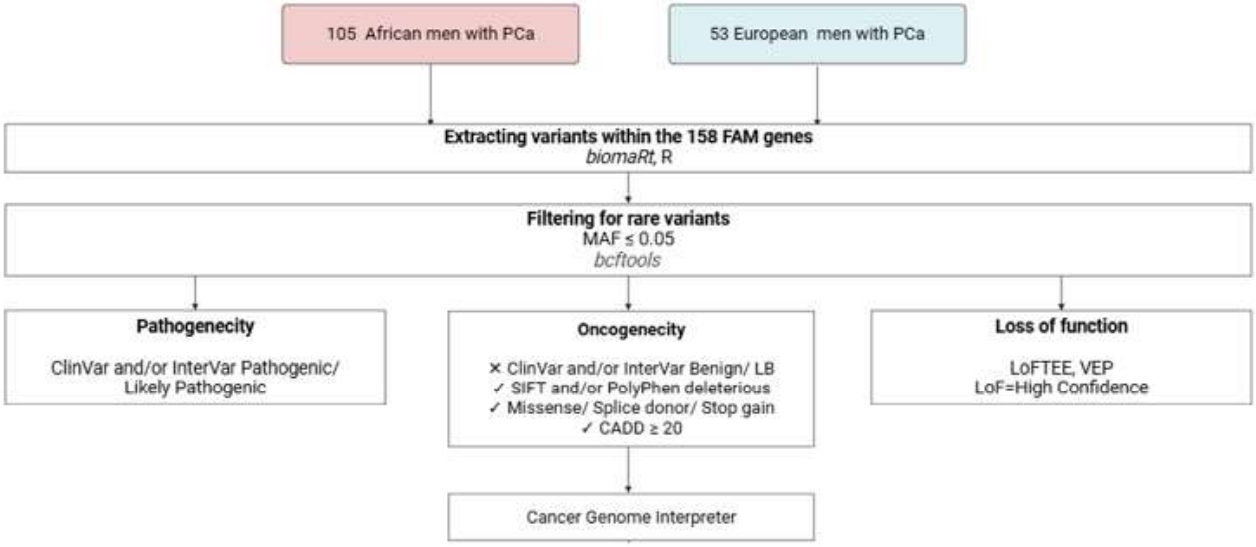


Figure 10: Assessment of rare germline variants for pathogenicity, oncogenicity and loss of function. VEP – variant effect predictor, LoFTEE - loss-of-function transcript effect estimator, CADD - combined annotation dependent depletion

3.2.3 Somatic variant analysis

Tumour mutational burden (TMB) was calculated for each patient by dividing the total number of somatic variants (SNVs and indels), by the total genome size of the 158 genes involved in FAM (8.17 Mbps) [194].

$$\text{Total genome size (Mbp)} = \frac{\sum(\text{end position} - \text{start position})}{1'000'000}$$

$$\text{TMB (for each patient)} = \frac{\text{SNVs} + \text{indels}}{\text{Total genome size}}$$

For functional impact prediction SIFT [182] and PolyPhen [183] scores were determined using the SNPnexus v.4 annotation tool [195]. Variants identified by SIFT as "deleterious" or "deleterious–low confidence" or identified by PolyPhen as "possibly damaging" or "probably damaging" were determined to be potentially damaging variants (PDVs). LOFTEE was used as a plugin within the Ensembl variant effect predictor (VEP) tool to enhance the annotation of loss-of-function (LoF)

variants [192]. Variants classified as "high confidence" were also determined to be PDVs. Lastly, to visualize patients' overall somatic variant landscape the maftools R package [196] was used to generate a summary oncoplot.

CHAPTER 4: RESULTS

4.1 COMMON GERMLINE VARIANT ANALYSIS

4.1.1 FAM associated PCa risk alleles

Of the 305 SNPs from the case-control study, nine SNPs were found to be significantly associated with PCa (figure 11), with the top three candidates including rs648831 (*BCKDHB*), rs2228063 (*CA2*) and rs45507397 (*FASN*). While none of the candidate alleles were pathogenic or likely pathogenic in ClinVar or InterVar (table 3), three variants were new to ClinVar and InterVar, including the top variant rs648831 (*BCKDHB*), rs652889 (*PTPRG*) and rs5751114 (*ACO2*). The pathogenicity of one variant was uncertain in InterVar and new to ClinVar. Further analysis for functionality identified three risk alleles as deleterious and/or damaging, including rs144668507 (*D2HGDH*), rs114864468 (*EHHADH*) and one of the top candidates rs2228063 (*CA2*).

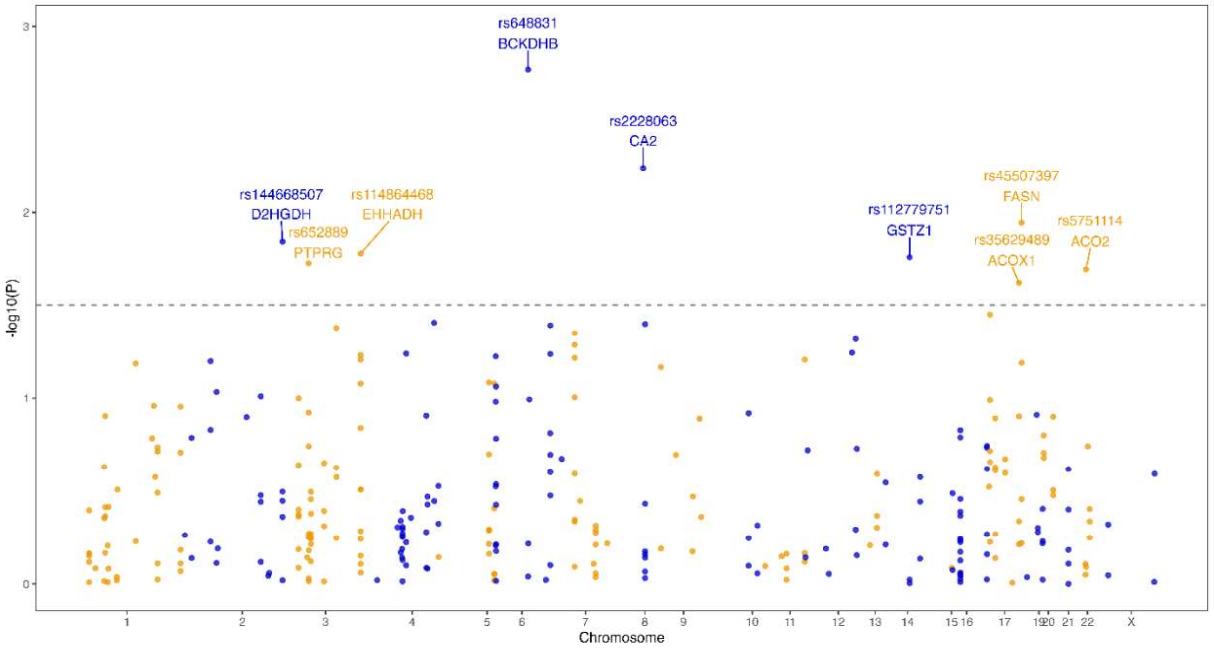


Figure 11: A Manhattan plot of -log10 p-values from a logistic regression analysis of 451 cases and 292 controls. The dashed line shows the significant threshold p-value of 0.05. The 9 variants above the threshold are significantly associated with PCa. Alternating orange and blue colours are used to distinguish adjacent chromosomes along the x-axis.

Table 3: Top associated significant variants from a logistic regression of cases (n=451) and controls (n=292).

CHR	SNP	BP	A1	A2	Gene	MAF	MAF_Cases	MAF_Controls	OR	P-value	InterVar	ClinVar	SIFT	PolyPhen	Cases (n)	Controls (n)
2	rs144668507	2.42E+08	T	C	*D2HGDH*	0.083	0.098	0.062	1.665	0.014	LB	B	D	B	82	34
3	rs652889	61808380	C	A	*PTPRG*	0.269	0.292	0.235	1.331	0.019	Novel	Novel	-	-	179	107
3	rs114864468	1.85E+08	C	G	*EHHADH*	0.039	0.049	0.024	2.136	0.017	B	B	D	PD	44	14
6	rs648831	80246491	T	C	*BCKDHB*	0.100	0.081	0.130	1.758	0.002	Novel	Novel	-	-	71	68
8	rs2228063	85480760	G	A	*CA2*	0.086	0.070	0.111	1.678	0.006	B	B	D	-	59	59
14	rs112779751	77331128	C	T	*GSTZ1*	0.047	0.0366	0.063	0.553	0.017	Uncertain	Novel	T	B	33	35
17	rs35629489	75948415	A	G	*ACOX1*	0.059	0.070	0.041	1.753	0.024	B	Novel	-	-	61	22
17	rs45507397	82081205	T	C	*FASN*	0.052	0.064	0.034	1.975	0.011	B	B	T	T	56	20
22	rs5751114	41484766	A	G	*ACO2*	0.310	0.333	0.274	1.309	0.020	Novel	Novel	-	-	188	121

Abbreviations: CHR-Chromosome, SNP-Single Nucleotide Polymorphism, BP-Base position, MAF-Minor Allele Frequency, OR-Odds Ratio, B-benign, T-tolerated, PD-Potentially damaging, D-damaging. Variants declared uncertain significance are those that do not meet the criteria for Pathogenic and Benign classification or are contradictory.

4.1.2 FAM risk alleles associated with aggressive PCa

Of the 303 SNPs from the high risk PCa (HRPCa) - vs low-risk PCa (LRPCa) study, eight SNPs were identified to be significantly associated with aggressive disease (Figure 12). Notably, two were found in the *INMT* gene (rs6970210 and rs6970605), with the latter variant being both deleterious and damaging. The other deleterious and damaging variant was the rs35097308 (*AOC3*) which is a top variant alongside two variants in chromosome 12; rs34441328 (*ERP29*) and rs2014355 (*ACADS*). As with the case-control analysis, no variants were pathogenic however one variant, rs34441328 (*ERP29*) was of uncertain significance in InterVar (Table 4).

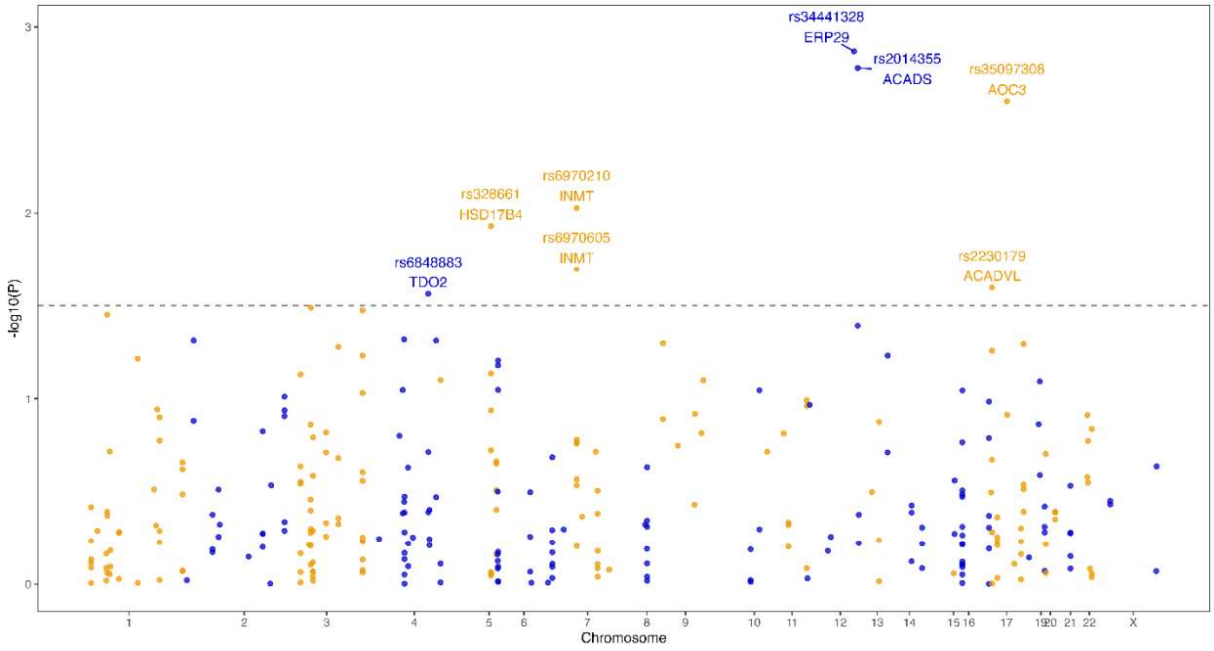


Figure 12: A Manhattan plot of -log10 p-values from a logistic regression analysis of 303 variants from 203 HRPCa vs 461 LRPCa. The dashed line shows the significant threshold p-value of 0.05. Eight variants were more associated with PCa as they had low p-values. The lower the p-value, the more the variant is associated with PCa. Alternating orange and blue colours are used to distinguish adjacent chromosomes along the x-axis.

Table 4: Top associated significant variants from a logistic regression of high risk prostate cancer cases (ISUP grade 3-5, n=203) and low risk prostate cancer cases or controls (ISUP grade 1-2/No PCa, n=461).

CHR	SNP	BP	A1	A2	Gene	MAF	MAF_HRPCa cases	MAF_LRPCa Controls	OR	P-value	INTERVAR	CLINVAR	SIFT	PolyPhen	Cases (n)	Controls (n)
4	rs6848883	1.56E+08	G	A	*TDO2*	0.452	0.406	0.472	0.7593	0.027	B	Novel	-	-	111	243
5	rs328661	1.2E+08	T	C	*HSD17B4*	0.260	0.217	0.278	0.6906	0.012	Novel	Novel	-	-	72	205
7	rs6970210	30755796	G	A	*INMT*	0.180	0.222	0.162	1.478	0.009	B	Novel	T	-	63	133
7	rs6970605	30755832	A	G	*INMT*	0.177	0.214	0.161	1.421	0.020	B	Novel	D	PD	66	134
12	rs34441328	1.12E+08	T	G	*ERP29*	0.038	0.064	0.026	2.566	0.001	Uncertain	Novel	T	B	26	25
12	rs2014355	1.21E+08	C	T	*ACADS*	0.059	0.091	0.045	2.113	0.002	Novel	B	-	-	37	39
17	rs2230179	7220033	T	C	*ACADVL*	0.032	0.049	0.025	2.008	0.025	B	B	T	B	18	24
17	rs35097308	42852455	C	T	*AOC3*	0.048	0.076	0.036	2.189	0.003	B	B	D	PD	27	34

Abbreviations: CHR-Chromosome, SNP-Single Nucleotide Polymorphism, BP-Base position, MAF-Minor Allele Frequency, OR-Odds Ratio, B- benign, T-tolerated, PD-Potentially damaging, D-damaging. Variants declared uncertain significance are those that do not meet the criteria for Pathogenic and Benign classification or are contradictory.

4.2 RARE GERMLINE VARIANT ANALYSIS

Focusing on 158 genes related to fatty acid metabolism (FAM), a total of 38,564 single nucleotide variants (SNVs) in 53 European men and 72,338 SNVs in 103 African men were interrogated. The SNVs were further filtered through three phases (figure 13). In phase I, variants with a minor allele frequency (MAF) ≤ 5% were retained, resulting in 19,957 SNVs for Europeans and 44,432 for Africans. In phase II, functional predictions using SIFT, CADD and PolyPhen identified 65 potentially damaging variants (PDVs) in Europeans and 132 in Africans. Finally, phase III filtered these PDVs using InterVar and ClinVar for pathogenicity, yielding 5 pathogenic or likely pathogenic variants in Europeans (7.7% of the initial PDVs, prevalence of 11.3%, 6/53 patients) and 10 pathogenic variants in Africans (7.6% of the initial PDVs; prevalence of 9.5%, 10/153 patients) (table 1). This shows a higher number but lower proportion of pathogenic variants in the African cohort compared to the European cohort.

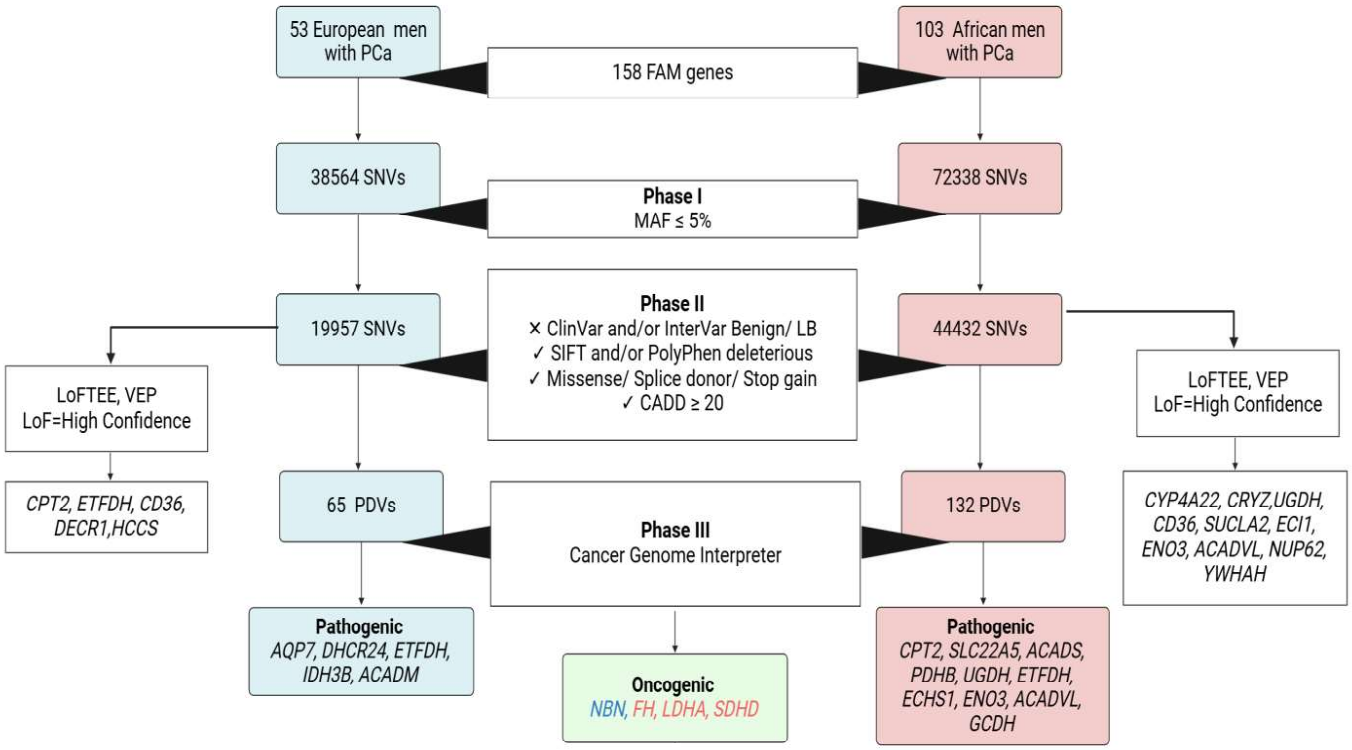


Figure 13. Comparative analysis of rare germline variants for pathogenicity, oncogenicity and loss of function. Genes in red contain variants found in African patients, while genes in blue contain variants found in European patients.

Abbreviations: PCa - Prostate cancer, FAM - fatty acid metabolism, MAF - minor allele frequency, LP - likely pathogenic, SNVs - single nucleotide polymorphisms, PDVs- potentially damaging variants

4.2.1 Rare pathogenic variants

Taking a closer look at the PDVs identified (Table 5), there was no overlap in pathogenic variants between ethnicities. Four of the African PDVs identified were novel, S5Y, F215Wfs*60 and Y108C in *PDHB, ACADVL* and *UGDH* respectively, and an unidentified variant in *ENO3*. Another variant in *ETFDH*, S491Ffs*2, was novel in Europeans and the most prevalent variant was G264V in *AQP7* found in 4.26% of the study cohort (2/53 Europeans). In this study, 80% and 83.3% of the African and European patients, respectively, with rare pathogenic variants presented with high risk PCa (ISUP ≥ 4). Follow-up clinical data was not available for the African cohort. Both patients with variants in *AQP7* had the most high-risk PCa (ISUP=4) with one patient presenting with boney metastasis and another with distant visceral metastasis (Table 6). Of all 16 patients with rare pathogenic germline variants, 62.5% were African patients, two of which had significantly high PSA levels 130 ng/ml and 92 ng/ml.

Table 5: Description of identified rare pathogenic germline variants

Variant ID	Gene	dbSNP	Variant type	Protein change	MAF (euro)	MAF (afro)	MAF in gnomad (euro)	MAF in gnomad (afro)
1:53210354:C/T	*CPT2*	rs74315298	missense	P227L	0	0.004762	8.47E-07	0.00104
chr5:132393687:C/T	*SLC22A5*	rs377216516	missense	R488C	0	0.004762	2.46E-05	2.67E-05
chr12:120737893:T/C	*ACADS*	rs57443665	missense	W177R	0	0.004762	3.39E-06	0.006663
chr3:58433796:G/T	*PDHB*	.	missense	S5Y	0	0.004762	0	2.67E-05
chr4:39510803:T/C	*UGDH*	.	missense	Y108C	0	0.004762	Variant not found	Variant not found
chr4:158708445:CAT/C	*ETFDH*	rs767795266	Stop-gain	p.C592*	0	0.004762	4.25E-06	4.01E-05
chr10:133369906:CAT/C	*ECHS1*	rs777218310	frameshift substitution	Y137Cfs*7	0	0.004762	8.48E-07	0.000427
chr17:4956683:T/G	*ENO3*	.	.	.	0	0.004762	Variant not found	Variant not found
chr17:7221630:GCT/G	*ACADVL*	.	frameshift substitution	F215Wfs*60	0	0.004762	Variant not found	Variant not found
chr19:12896934:G/A	*GCDH*	rs121434371	missense	A293T	0	0.01429	2.46E-05	0.00032
chr1:54871495:G/A	*DHCR24*	rs138667252	missense	P244L	0.009434	0	0.001163	0.000294
chr1:75761161:A/G	*ACADM*	rs77931234	missense	K329E	0.009434	0	0.00748	0.001212
chr4:158706629:G/GT	*ETFDH*	.	frameshift substitution	S491Ffs*2	0.009434	0	1.36E-05	0

chr9:33385243:C/A	*AQP7*	rs62542743	missense	G264V	0.01887	0	0.05245	0.007964
chr20:2660738:G/A	*IDH3B*	rs185838502	stop gain	R164X	0.009434	0	4.83E-05	0

Abbreviations: MAF - minor allele frequency, dbSNP - single nucleotide polymorphisms database.

Table 6: Clinical data for PCa patients with rare pathogenic variants.

Sample Id	**Ancestry**	**Variant ID**	**Gene**	**Age**	**PSA (ng/ml)**	**ISUP_G**	**Outcome**
UP2351	African	chr12:120737893:T/C	*ACADS*	65-69	92	5	Unknown
UP2213	African	chr17:7221630:GCT/G	*ACADVL*	65-69	25.8	5	Unknown
UP2101	African	1:53210354:C/T	*CPT2*	55-59	75	5	Unknown
UP2372	African	chr10:133369906:CAT/C	*ECHS1*	65-69	37.4	5	Unknown
KAL0004	African	chr17:4956683:T/G	*ENO3*	70-74	30.57	NA	Unknown
UP2099	African	chr4:158708445:CAT/C	*ETFDH*	75-79	100	4	Unknown
UP2116	African	chr19:12896934:G/A	*GCDH*	95-99	65	5	Unknown
UP2159	African	chr3:58433796:G/T	*PDHB*	65-69	11.9	4	Unknown
SMU079	African	chr5:132393687:C/T	*SLC22A5*	65-69	21.58	2	Unknown
UP2187	African	chr4:39510803:T/C	*UGDH*	65-69	130	4	Unknown
11926	European	chr1:75761161:A/G	*ACADM*	55-59	16	5	PSA alone
14365	European	chr9:33385243:C/A	*AQP7*	60-64	7.2	5	PSA + boney metastasis
16677	European	chr9:33385243:C/A	*AQP7*	65-69	6.9	5	PSA + distant visceral metastasis
16995	European	chr1:54871495:G/A	*DHCR24*	50-54	8.1	4	PSA alone
12543-1081384	European	chr4:158706629:G/GT	*ETFDH*	50-54	18.6	5	PSA alone
11452-1056021	European	chr20:2660738:G/A	*IDH3B*	65-69	11	1	Nil relapse at last follow-up

Abbreviations; PSA - prostate specific antigen, ISUP_G - International Society of Urological Pathologist Grade.

4.2.2 Rare oncogenic variants

There were only four oncogenic variants (OVs) identified in this study, including a novel variant K317N in *LDHA* (Table 7). Three of the four variants were found in African patients. Two OVs had a prevalence of 1.9%, V435M in *FH* and Y93C in *SDHD* (Table 8) and 83% (5/6) of all patients with oncogenic variants had high-risk PCa (ISUP_G ≥ 4).

Table 7: Description of identified rare oncogenic germline variants.

Variant ID	Gene	dbSNP	Variant type	Protein change	MAF (euro)	MAF (afro)	MAF in gnomad (euro)	MAF in gnomad (afro)
chr8:89971232:G/A	*NBN*	rs34767364	missense	R215W	0.009434	0	0.003791	0.0004804
chr1:241500524:C/T	*FH*	rs147528200	missense	V435M	0	0.009524	5.85E-05	0.0005224
chr11:18407233:G/T	*LDHA*	.	missense	K317N	0	0.004762	Variant not found	Variant not found
chr11:112088975:A/G	*SDHD*	rs142135772	missense	Y93C	0	0.009524	3.39E-06	0.001359

Abbreviations: MAF- Minor Allele Frequency, dbSNP- Single Nucleotide Polymorphisms database.

Table 8: Clinical data for patients with rare oncogenic variants.

Sample Id	Ancestry	Variant ID	Gene	Age	PSA (ng/ml)	ISUP_G	TMB (whole genome)	TMB (FAM)	Outcome
N0077	African	chr1:241500524:C/T	*FH*	70-74	49.3	4	0.19592	0	Unknown
UP2050	African	chr1:241500524:C/T	*FH*	65-69	581	5	3.003562	2.936148	Unknown
SMU029	African	chr11:18407233:G/T	*LDHA*	60-64	19.95	1	0.534974	0.489358	Unknown
N0078	African	chr11:112088975:A/G	*SDHD*	65-69	>1500	5	1.192034	0.734037	Unknown
TSH005	African	chr11:112088975:A/G	*SDHD*	55-59	100	4	0.927461	0.489358	Unknown
16176	European	chr8:89971232:G/A	*NBN*	60-64	8.5	5	1.305376	1.101056	Nil relapse at last follow-up

Abbreviations; PSA- Prostate Specific Antigen, ISUP_G- International Society of Urological Pathologist Grade, TMB- Tumour Mutational Burden

4.2.3 Rare variants with predicted loss of function (LoF)

Fifteen LoF variants were identified in this study with four novel variants in *SUCLA2, ENO3, ACADVL* and *ETFDH* (Table 9). Two variants in *CD36* were found, rs571975065 and rs397811824, with the latter being present in both the European and African cohort (Table 10). Two novel variants in *ETFDH* and *ENO3* were both pathogenic and had loss of function.

Table 9: Loss of function variants

Variant ID	Gene	rsID	MAF african gnomad	MAF euro gnomad	MAF Afro	MAF Euro
1:47143917:G/T	*CYP4A22*	rs112811686	0.01476	1.95E-05	0.009524	0
1:74707102:C/T	*CRYZ*	rs6668086	0.005513	2.63E-06	0.014286	0
4:39509908:C/CT	*UGDH*	rs111436071	7.17E-05	0	0.009524	0
7:80656572:TA/T	*CD36*	rs571975065	0.0162	1.78E-05	0.019048	0
7:80674144:ATAAG/A	*CD36*	rs397811824	0.01562	0.01494	0.02381	0.009434
13:47971885:T/C	*SUCLA2*	novel	Variant not found	Variant not found	0.004762	0
16:2243317:C/A	*ECI1*	rs200630484	0.001054	4.07E-05	0.019048	0
17:4956683:T/G	*ENO3*	novel	Variant not found	Variant not found	0.004762	0
17:7221630:GCT/G	*ACADVL*	novel	Variant not found	Variant not found	0.004762	0
19:49908973:C/T	*NUP62*	rs113473061	0.01439	6.72E-05	0.014286	0
22:31950316:T/TA	*YWHAH*	rs2093841510	3.38E-05	0	0.004762	0
1:53211252:T/C	*CPT2*	rs113493395	0.000693	0.003703	0	0.009434
4:158706629:G/GT	*ETFDH*	novel	0	1.36E-05	0	0.009434
8:90006279:T/A	*DECR1*	rs16902273	0.1632	0.03091	0	0.037736
X:11120047:T/C	*HCCS*	rs755537648	0.000293	0.001913	0	0.018868

The most prevalent variants from the African cohort were rs397811824 and rs200630484 in CD36 and ECI1 respectively. Both variants had a prevalence of 4.8% (5/105). In the European cohort, the most prevalent variant was rs16902273 in DECR1 with a prevalence of 7.5% (4/53). Interestingly, all patients with this variant had high risk PCa (ISUP_G = 5). Compared to the pathogenic and oncogenic variants, the LoF variants were the most prevalent. The LoF variants were present in 16.5% and 15% of the European and African cohorts respectively. 73.5% of the patients with LoF variants had high risk PCa (ISUP_G ≥ 4).

Table 10: Clinical data for patients with LoF variants

Variant ID	Gene	Sample_id	Country	Age	PSA	ISUP_GG	TMB	Outcome
chr1:47143917:G/T	*CYP4A22*	KAL0101	SA	70-74	32.39	5	0.070596	Unknown
chr1:47143917:G/T	*CYP4A23*	N0059	SA	75-79	153	5	0.267811	Unknown
chr1:74707102:C/T	*CRYZ*	SMU080	SA	60-64	23.31	4	1.34715	Unknown
chr1:74707102:C/T	*CRYZ*	TSH003	SA	75-79	41	2	0.169042	Unknown
chr1:74707102:C/T	*CRYZ*	UP2004	SA	70-74	100	5	1.020402	Unknown
chr4:39509908:C/CT	*UGDH*	UP2004	SA	70-74	100	5	1.020402	Unknown
chr7:80656572:T/AT	*CD36*	N0067	SA	65-69	1580	4	2.052461	Unknown
chr7:80656572:T/AT	*CD36*	SMU032	SA	65-69	50	NA	1.214054	Unknown
chr7:80656572:T/AT	*CD36*	SMU064	SA	70-74	13.68	3	0.189119	Unknown
chr7:80656572:T/AT	*CD36*	UP2115	SA	55-59	21	4	0.090674	Unknown
chr7:80674144:ATAAG/A	*CD36*	KAL0054	SA	60-64	42.93	5	0.685233	Unknown
chr7:80674144:ATAAG/A	*CD36*	SMU027	SA	65-69	88.06	5	1.544365	Unknown
chr7:80674144:ATAAG/A	*CD36*	SMU029	SA	60-64	19.95	1	0.534974	Unknown
chr7:80674144:ATAAG/A	*CD36*	SMU030	SA	65-69	193.66	1	1.877267	Unknown
chr7:80674144:ATAAG/A	*CD36*	UP2048	SA	55-59	8.21	5	0.049223	Unknown
chr13:47971885:T/C	*SUCLA2*	UP2159	SA	65-69	11.9	4	0.202396	Unknown
chr16:2243317:C/A	*ECI1*	KAL0043	SA	80-84	2736/68?	5	3.029469	Unknown
chr16:2243317:C/A	*ECI1*	N0081	SA	60-64	1232.8	5	1.134715	Unknown
chr16:2243317:C/A	*ECI1*	SMU089	SA	50-54	18.38	3	0.965674	Unknown
chr16:2243317:C/A	*ECI1*	SMU157	SA	60-64	33.5	4	0.0557	Unknown
chr16:2243317:C/A	*ECI1*	KAL0004	SA	70-74	30.57	NA	1.193005	Unknown

chr17:7221630:GCT/G	*ACADVL*	UP2213	SA	65-69	25.8	5	0.557319	Unknown
chr19:49908973:C/T	*NUP62*	N0085	SA	70-74	137	4	1.00842	Unknown
chr19:49908973:C/T	*NUP62*	SMU107	SA	70-74	53.1	1	0.946244	Unknown
chr19:49908973:C/T	*NUP62*	TSH005	SA	55-59	100	4	0.927461	Unknown
chr22:31950316:T/TA	*YWHAH*	UP2267	SA	65-69	4847	4	2.28886	Unknown
chr7:80674144:ATAAG/A	*CD36*	11129	AU	60-64	3.7	5	1.490933	PSA + boney metastasis
chr1:53211252:T/C	*CPT2*	17135	AU	60-64	7.79	5	0.026231	PSA alone
chr4:158706629:G/GT	*ETFDH*	12543	AU	50-54	18.6	5	1.019106	PSA alone
chr8:90006279:T/A	*DECR1*	11099	AU	70-74	9.9	5	0.121438	PSA + local
chr8:90006279:T/A	*DECR1*	11119	AU	65-69	8	5	0.028821	Nil relapse at last follow-up
chr8:90006279:T/A	*DECR1*	11830	AU	60-64	3.5	5	1.257124	Nil relapse at last follow-up
chr8:90006279:T/A	*DECR1*	13104	AU	55-59	4.7	5	1.522668	PSA + boney metastasis
chrX:11120047:T/C	*HCCS*	5902	AU	45-49	6.7	1	0.628562	Nil relapse at last follow-up

Abbreviations; PSA- Prostate Specific Antigen, ISUP_G- International Society of Urological Pathologist Grade, SA- South African, AU- Australia

4.3 SOMATIC VARIANT ANALYSIS

4.3.1 Tumour mutational burden

Within the study cohort, 17% of European-derived tumours and 12% of African-derived tumours lacked alterations within the FAM pathway. The boxplot in Figure 14 shows the distribution and variability of TMB across the European and African population. Although the African cohort had a higher mean TMB than the European cohort, the difference was not statistically significant, (p-value 0.056). Both cohorts also had a median TMB of 0.86 mutations/Mb (mut/Mb).

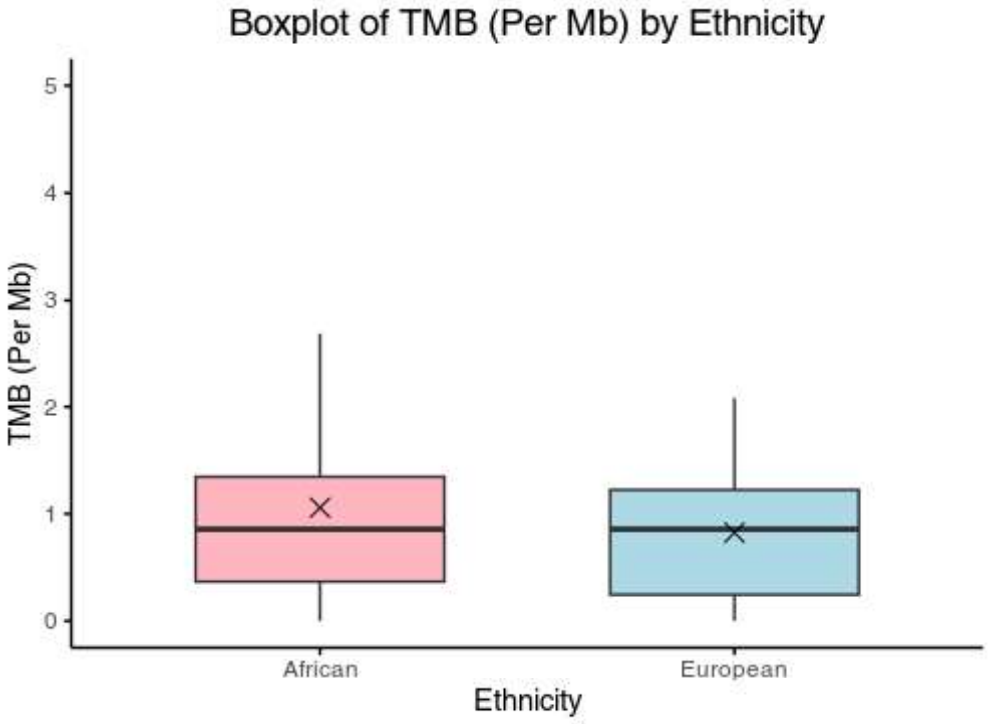


Figure 14: Box plot of tumour mutational burden (TMB). The European patients had a mean TMB of 0.824 mut/Mb while the African patients have a mean TMB of 1.218 mut/Mb.

4.3.2 Functional impact prediction

Potentially damaging variants (PDVs) were identified in both the European and African derived tumours, (figure 15 and Table 11). In the European cohort, only 0.9% of the SNVs were PDVs (Table 12) and only 5.7% of the European-derived tumours harboured PDVs. For the African cohort, 0.5% of the SNVs were PDVs, and they were present in only 4.8% of African-derived tumours (Table 12). There was no loss of function variants found in the African-derived tumours but there was one loss of function variant in *FMO1* found in an European derived tumour.

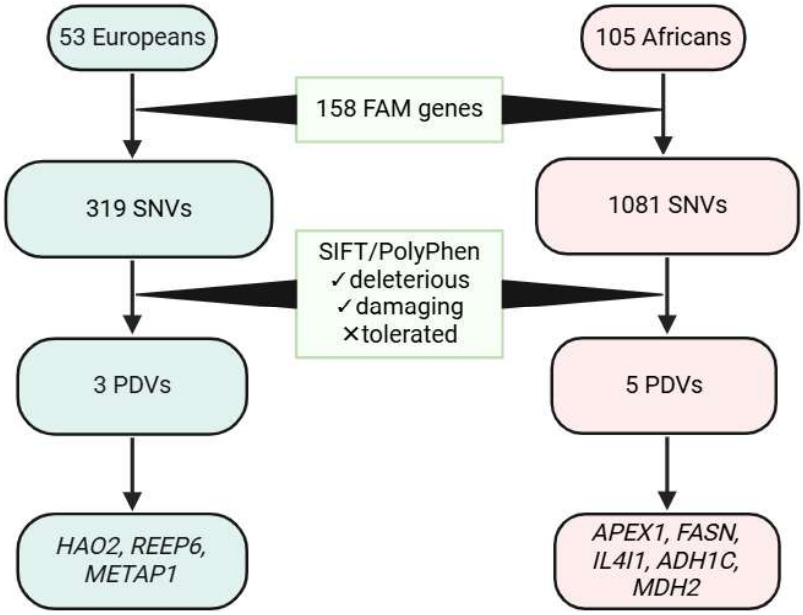


Figure 15: Functional impact prediction using SIFT and PolyPhen. Variants that were predicted to be deleterious and/or damaging were called potentially damaging variants (PDVs). Three PDVs in HAO2, REEP6 and METAP1 were found in European-derived tumours and five PDVs were found in APEX1, FASN, IL4I1, ADH1C and MDH2 in African-derived tumours.

Table 11: Potentially damaging variants identified from 53 European-ancestry derived tumours and 105 African-ancestry derived tumours.

Gene	Chr	Position	Ref	Alt	dbSNP
HAO2	1	1.19E+08	A	G	None
ADH1C	4	99342809	G	A	rs1395450124
METAP1	4	99034338	C	T	None
MDH2	7	76060421	G	T	None
APEX1	14	20457260	C	T	rs375526265
FASN	17	82085634	C	A	None
IL4I1	19	49890568	A	G	None
REEP6	19	1491289	G	T	None
FMO1	1	1.71E+08	CTTCTCTTTTATTTTCTATAGGTATTAATGCCTT	-	None

Chr - chromosome, Ref - reference allele, Alt - alternative allele, dbSNP- single-nucleotide polymorphism database

Table 12: Clinical data of patients with potentially damaging variants.

Gene	**Patient**	**Ethnicity**	**ISUP_G**	**PSA**	**Age**	**TMB**	**Clinical outcome**
APEX1	N0087	African	4	12	60-64	1.10	N/A
FASN	KAL0043	African	5	Unknown	80-85	3.03	N/A
IL4I1	KAL0072	African	5	53.25	60-64	15.66	N/A
ADH1C	SMU089	African	3	18.38	50-54	0.97	N/A
MDH2	UP2116	African	5	65	95-99	2.10	N/A
HAO2	16311	European	5	16.5	60-64	0.87	PSA + nodal metastasis
REEP6	14862	European	5	12.40	60-64	1.42	Nil relapse at last follow-up
METAP1	16599	European	4	8.10	50-54	1.84	PSA alone
FMO1	15178	European	4	6.7	60-64	1.03	PSA + boney + distant visceral metastasis

PSA - prostate specific antigen, ISUP_G - International Society of Urological Pathologist Grade. Notably, all patients had aggressive PCa, characterized by ISUP 3-5.

4.3.3 Somatic variant landscape

The oncoplot in Figure 16 shows the top 20 mutated genes. Of the top mutated genes, only four (*FASN, ACADL, ACOT8,* and *ADH1C*) were present in more than one patient and all four except *FASN* exclusive to African-derived tumours. The oncoplot also shows that European patients had significantly lower PSA levels than African patients.

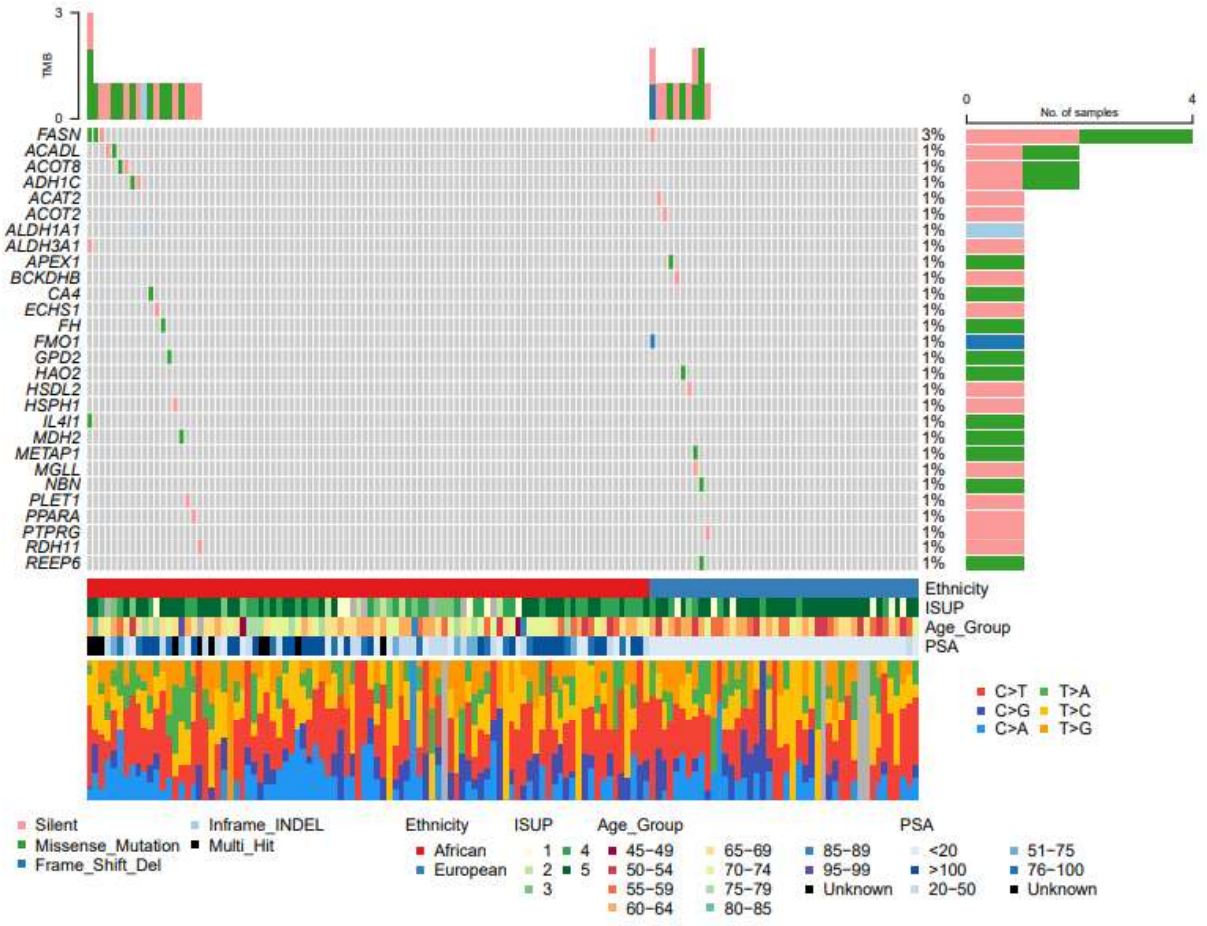


Figure 16: An oncoplot showing the somatic alteration landscape. The top bar graph labelled TMB shows the number of somatic alterations per tumour. The middle gene panel shows synonymous and non-synonymous variants in a maximum of 20 top altered genes. The bottom panels annotate patient ethnicities, ISUP grades, age groups, and PSA groups. The right-hand bar graph displays mutation frequencies.

CHAPTER 5: DISCUSSION

Recent advancements in prostate cancer research have included the incorporation of diverse genomic data. This has significantly expanded the understanding of genetic risk factors. The inclusion of African genomic data in meta-analysis of GWAS increased the known common germline PCa risk variants by 47% in 2021 (from 183 to 269) [89], and then in early 2023, the number of variants increased by a further 3.3% to 278 [197]. By the end of 2023, the number of known PCa risk variants had catapulted by 62.2% (to 451) [198]. Another study in 2023 found 17 SNPs significantly associated with PCa and 25 SNPs associated with high risk PCa [176]. The latest study from 2024 identified 15 new significant SNPs in African men [199]. Of these known variants, three are located within genes of the fatty acid metabolism pathway, including *BCKDHB* (rs146575246), *MDH1* (rs139283528) and *ELOVL5* (rs594260) showing the need to investigate the FAM pathway. Absent from the 451 known PCa risk loci, this study highlights 17 common germline variants within FAM genes as potential candidates for PCa risk or associated aggressive disease. As such, this study highlights the need for further interrogation of the FAM pathway which bodes to be a critical metabolic network in PCa.

In the case control study, the novel variant rs648831(*BCKDHB*) was the most associated with PCa risk, p=0.002. Although no information is available in relation to PCa, this variant has previously been associated with height [200], breast cancer [201] and osteoarthritis [202]. Specific pathogenic variants in the branched chain keto acid dehydrogenase E1 subunit beta (*BCKDHB*) gene cause the fatal, inborn Maple Syrup Urine disease where patients are unable to breakdown process certain amino acids [203]. *FASN,* encoding for fatty acid synthase was another one of the top associated variants (p=0.011), alterations to this gene is no surprise because overexpression of *FASN* has been associated with prostatic tumorigenesis [204]. The role of *FASN* in PCa has been studied extensively and has led to the development of therapeutic treatments that use compounds that inhibit *FASN* such as alpinumisoflavone [205] and orlistat [206].

Of the nine significant PCa risk alleles identified in the case control study, only three of them were found to have deleterious or damaging potential by SIFT and/or PolyPhen. This includes the top associated rs2228063 (*CA2*), p=0.006. The role of carbonic anhydrase II (*CA II*) in PCa progression has not been extensively studied compared to the well-researched roles of carbonic anhydrases 4 and 7 (*CA4* and *CA7*) [207–211]. *CA2* encodes a tumour associated protein whose overexpression is associated with long non-coding RNAs [212]. The deleterious rs114864468 variant in the D-2-hydroxyglutarate dehydrogenase (*D2HGDH)* gene affects the conversion of D2HG to α-ketoglutarate, impacting cancer progression [213]. *D2HGDH* overexpression boosts anti-tumour activity and immune response [213, 214]. The role of *D2HGDH* in cancer is conflicting as it serves as a biomarker in breast cancer [215], where higher expression correlates with malignancy but reduces malignancy risk in colorectal cancer [216]. It is also part of prognostic markers for head and neck squamous cell carcinoma (HNSCC) and ovarian cancer [217, 218]. The *D2HGDH-GAL3ST2* fusion gene is more common in PCa, especially in African Americans, and silencing it reduces cell growth and motility [219].

The only variant that was both deleterious and damaging, rs114864468 *(EHHADH)*, encodes for enoyl-CoA hydratase and 3-hydroxyacyl CoA dehydrogenase, a member of the 3-hydroxyacyl-CoA dehydrogenase family which plays a role in the fatty acid beta-oxidation

pathway by catalyzing the degradation of long-chain dicarboxylic acids [220]. The functions of *EHHADH* in fatty acid metabolism can be performed by a similar enzyme *HSD17B4*, suggesting that *EHHADH* is redundant [221]. This has been supported by lack of defective phenotypes in *EHHADH* knockout mice. On the contrary, reduced expression of *EHHADH* is linked to reduced hepatocellular carcinoma (HCC) and bladder cancer progression, suggesting its potential as a therapeutic target [222]. EHHADH has been extensively studied in relation to liver cancer, resulting in the development of a risk assessment model for liver cancer prognosis [223–225]. In PCa, *EHHADH* was significantly overexpressed in immunohistochemical analyses, leading to the development of a four-gene diagnosis model [226].

Only eight out of 303 SNPs were significantly associated with high risk PCa and only two were deleterious and potentially damaging, rs6970605(*INMT*) and rs35097308(*AOC3*). Indolethylamine-N-methyltransferase (*INMT*) converts inositol into inositol monophosphate, a precursor of inositol triphosphate (IP3) that regulates calcium channels which regulate the activity of CPT1 and SREBPs [227]. In CRPC, *INMT* expression is elevated and linked to poor prognosis, promoting resistance via detoxification of anticancer metabolites [23]. Conversely, analysis of The Cancer Genome Atlas (TCGA) indicates that lower *INMT* levels in PCa are associated with worse clinical outcomes, suggesting that *INMT* may inhibit tumour progression by enhancing immune response [228]. Furthermore, β-hydroxybutyrate has been found to inhibit PCa malignancy by suppressing *INMT* expression through β-hydroxybutyrylation, reducing the stem-like characteristics of cancer cells [229]. The other deleterious variant rs35097308(*AOC3*) is highly associated with aggressive PCa, p=0.003. *AOC3* encodes for amine oxidase copper-containing 3, also known as vascular adhesion protein-1 (*VAP-1*), which regulates uptake of long-chain fatty acids [230]. *AOC3* is significantly downregulated in TGF-β1-treated fibroblasts, making it a potential marker of colorectal cancer (CRC) progression [231]. Additionally, *AOC3* has been identified as an autophagy-related biomarker with significant prognostic value in CRC, highlighting its role in the interplay of cell death pathways [223]. Low stromal *AOC3* levels are linked to poor prognosis in breast cancer patients [233, 234]. A risk model including *AOC3* and three other genes (*CDK6, COL22A1,* and *RNASE6*) has shown remarkable prognostic value for survival rates and metastasis in osteosarcoma, suggesting its potential for therapy guidance [235].

In addition to *AOC3*, the variants rs34441328 (*ERP29*) and rs2014355 (*ACADS*) were also highly associated with aggressive PCa in the high-risk vs low risk study, p=0.001 and p=0.002 respectively. ERP29 is a novel endoplasmic reticulum protein that plays a key role in protein unfolding and secretion [236]. Downregulation of *ERP29* promotes metastasis and invasiveness of gastric cancer cells [237] while its upregulation inhibits metastasis of colorectal cancer [238]. In PCa, upregulating *ERP29* using dihydrotestosterone, and the tumour suppressor EPI-001 may have anti-tumour properties. Acyl-CoA dehydrogenase short-chain (*ACADS*) is a key enzyme in the mitochondria [239]. It is involved in the initial steps of fatty acid beta-oxidation pathway. In cancer, *ACADS* has been downregulated in colorectal cancer [240] and Pancreatic ductal adenocarcinoma [241] but upregulated overexpressed in hepatocarcinoma [242]. The expression of *ACADS* is regulated by DNA methylation in colorectal cancer and in hepatocarcinoma, making it a potential therapeutic target [240, 242].

While the analysis of common germline variants offers insight into inherited risk factors shared across populations, exploring rare germline variants allows for the identification of population-specific or high-impact genetic contributors to PCa susceptibility. The inclusion of FAM genes in this study revealed 16 rare pathogenic variants, highlighting the potential significance of this pathway. In this study, 13 out of 16 (81.3%) patients harbouring rare pathogenic variants in

FAM genes presented with aggressive PCa (ISUP ≥ 4), confirming the high penetrance within these rare variants. Notably, the rare pathogenic variant rs62542743 in the AQP7 gene was the most prevalent, observed in 4.26% of the cohort. Both patients with this variant had high-risk PCa (ISUP = 5), with one patient exhibiting bony metastasis and the other distal visceral metastasis. These findings suggest that AQP7 could be a relevant addition to germline testing panels, although this variant was found only in patients of European descent. Aquaporins (AQPs), which facilitate the regulated transport of glycerol, are gaining attention as potential targets for anticancer therapies [243]. Several AQPs, including AQP4, AQP7, AQP8, AQP10, and AQP11, are overexpressed in both benign and malignant human prostate tissues at the transcript level [243]. A study by Kushwaha et al., (2023) suggests a positive association between these AQPs and the initiation, progression, and recurrence of PCa post-therapy [244]. Further research is needed to establish AQPs as prognostic biomarkers for PCa, but these initial findings highlight their potential as targets in both diagnostics and therapeutics [244, 245].

The rare P277L variant in the *CPT2* gene was the only variant classified as pathogenic by both ClinVar and InterVar, highlighting the critical need for standardized methods to predict the pathogenicity of genetic variants. Another rare variant of *CPT2*, rs113493395, was found to have loss of function. *CPT2* plays a key role in fatty acid oxidation (FAO), and a study by Zimmer et al., (2016) suggests that PCa cells may increasingly rely on *CPT2* enzymes for growth when cyclin-dependent kinase 9 (CDK9) is inhibited [246]. This points to a potential therapeutic strategy that combines *CPT2* inhibition with CDK9 inhibitors in treating PCa. Moreover, *CPT2* expression has been reported to correlate significantly with poor prognosis and therapy resistance in PCa, suggesting that it could serve as a predictive biomarker [247, 248]. Therefore, the distribution of *CPT2* expression in cancer cells might be a valuable indicator for predicting therapeutic outcomes and guiding treatment strategies.

Novel pathogenic variants were found in UGDH, *ACADVL, ENO3, PDHB* and *ETFDH. ACADVL* codes for very long-chain specific acyl-CoA dehydrogenase, an enzyme involved in long chain fatty acid oxidation. *ACADVL* is upregulated in aggressive PCa tumours [249, 250] and its upregulation is associated with reduced survival [251]. Enolase 3 (*ENO3*) plays an important role in glycolysis but unlike other enolases (*ENO1* and *ENO2*), its role in PCa has not been studied. *ENO3* was classified as an oncogene in colorectal cancer [252] and a tumour suppressor in hepatocellular carcinoma [253]. The same novel pathogenic variant in *ENO3* was also identified in this study as a LoF variant, similar to a novel European variant in *ETFDH* that was also both pathogenic and had LoF. There were two variants in *ETFDH*, rs767795266 from the African cohort and a novel variant from the European cohort. The electron-transferring-flavoprotein dehydrogenase (*ETFDH*) has not been studied in oncology. The rare novel variant Y108C in *UGDH* was identified in a patient with high-risk PCa (ISUP=4) and an extraordinarily high PSA level of 130 ng/ml, which is significantly higher than the normal PSA level of 4 ng/ml**.** UGDH is involved in glucuronidation, a process that controls androgen levels in the prostate [254]. Overexpression of *UGDH* has been linked to increased androgen-independent growth and CRPC, suggesting that *UGDH* could be a valuable biomarker and therapeutic target in advanced PCa [255, 256, 256]. The other novel variant was in *PDHB*, a gene that has been associated with cervical cancer progression [257] and has been identified as a biomarker for renal clear cell carcinoma [258] but with no associations to PCa.

According to the NCCN, variants of uncertain significance (VUS) should not be used to inform clinical management [102]. However, as genomic databases grow, reclassification of VUS can refine patient diagnoses and precision therapies [259, 259]. In this study, VUS were further interrogated to identify oncogenic variants or variants with loss of function. Four oncogenic

variants were identified in *NBN, FH, SDHD* and *LDHA*. *NBN*, a well-known DNA damage repair gene that codes for nibrin protein, is included in the NCCN Clinical Practice guidelines for germline testing panels for PCa [1–3]. Although germline alterations in *NBN* correlate with increased PCa progression [1], several studies evaluating the R215W variant in *NBN* conclude that there is no association with PCa [1–3] or breast cancer [1]. Both fumarate hydratase (*FH*) and succinate dehydrogenase (*SDHD*) act as a tumour suppressor by metabolizing fumarate and succinate respectively, whose accumulation leads to activation of hypoxia inducible factors (HIFs) - transcriptional factors that promote angiogenesis and tumour growth [1, 2]. At abnormal levels fumarate undergoes a post-translational modification called succination, providing a diagnostic marker for *FH*-deficiency and tumour progression [260]. In PCa, the natural compound alternol increases *FH* activity to inhibit tumour progression [1] and the oncoprotein TRIM25 downregulates the expression of *FH* [1]. The role of lactate dehydrogenase A (*LDHA*) in PCa is well studied, with several studies labelling it a potential target for PCa treatment [248, 261–263].

In addition to pathogenic and oncogenic rare germline variants, this study also uncovered 16 variants with predicted loss of function. *CD36* harboured two rare loss-of-function (LoF) variants: rs571975065, which was exclusive to the African cohort, and rs397811824, which was the only variant shared across both cohorts. *CD36* codes for a fatty acid transporter that is upregulated in PCa tumours. Its deletion slows down tumour progression [264]. Conversely, succinate CoA ligase (*SUCLA2*), which has a novel LoF variant, is downregulated in PCa [265] and it is associated with CRPC neuroendocrine progression [266]. Dysregulation of genes like *SUCLA2* and *LDHA* involved in the TCA cycle affects the production of acetyl-CoA, the key building block for lipid synthesis [267]. The LoF variant, rs16902273, in *DECR1* was present in 50% (4/8) of all European patients with LoF variants and interestingly, all patients with the variant presented with aggressive PCa (ISUP grade group = 5). 2,4-dienoyl-CoA reductase (*DECR1*), is involved in the oxidation of PUFAs [268]. Silencing or knockdown of *DECR1* inhibits PCa and CRPC progression by promoting oxidative stress caused by accumulation of poly unsaturated fatty acids [268–270]. In the African cohort, the most prevalent LoF variant was rs200630484 in *ECI1* found in 19.2% (5/26) patients with LoF variants, 60% of the 5 patients had ISUP $\geq$ 4. Δ3, Δ2-enoyl-CoA isomerase 1 (*ECI1*) overexpression is associated with increased PCa growth and increased risk of biochemical recurrence due to its role in unsaturated fatty acid oxidation [271].

This study identified rare novel pathogenic variants in men of African-ancestry, similar to other studies, such as one conducted in Uganda on 651 PCa cases [272]. In the Ugandan cohort, rare pathogenic variants in *BRCA2, ATM, PALB2*, and the FAM gene *NBN* were significantly associated with a high risk of aggressive disease, highlighting the potential significance of the FAM pathway genes in the African context. The differences in occurrence and frequency of rare germline variants across different ethnicities highlights the importance of sequencing efforts in genetically diverse populations.

Building on the insights from rare germline variation, examining somatic mutations provides a comprehensive view of tumour-specific alterations that may interact with inherited predispositions to influence disease progression and disparity. The results of this study revealed that while African-derived tumours exhibited a higher mean TMB (1.218) compared to European-derived tumours (0.824), this difference was not statistically significant (p-value 0.056). This suggests that although there may be a trend towards higher genomic instability in African tumours, further investigation with larger sample sizes may be necessary to draw definitive conclusions. African Americans have been reported to have a higher rate of somatic

variants and unique variants in the PCa tumours as compared to Caucasians, further emphasizing the aggressiveness of PCa in men of African-ancestry [273]. Moreover, the identification of potentially damaging variants (PDVs) showed that both cohorts had low frequencies of PDVs, 0.9% in European-derived tumours and 0.5% in African-derived tumours, suggesting that the FAM pathway is highly conserved in PCa tumours. Notably, all patients that had PDVs had aggressive PCa characterized by high ISUP grades (3-5), highlighting the severity of PDVs in the FAM pathway.

Five PDVs were identified from African-derived tumours, including variants in *APEX1, MDH2, IL4I1, ADH1C,* and *FASN.* The *APEX1* gene is responsible for encoding apurinic/apyrimidinic endonuclease which functions primarily as an endogenous DNA repair enzyme but also inhibits lipid peroxidation, thereby promoting PCa tumour survival [274, 275]. Variants in the *APEX1* gene, including R237C found in this study, were previously identified in 15% of endometrial tumours, suggesting a role in cancer susceptibility [276]. The R237C variant was associated with decreased endonuclease activity and reduced ability to interact with enzymes acting downstream of *APEX1* in the base excision repair pathway, further supporting the role of *APEX1* variants in cancer susceptibility [274]. On the contrary, a study by Illuzzi et al., (2017) [277] demonstrated that overexpression of the R237C variant in a mouse cell line did not affect proliferation or cellular transformation, undermining its function as a cancer driver. The R237C variant has not been previously associated with PCa but other variants in *APEX1* including rs1130409 and rs1760944 have been associated with the development of PCa (Chen et al., 2016; Gong et al., 2021). Additionally, *APEX1* was shown to be upregulated in PCa tumours, making it a potential prognostic marker [281].

Another gene that has been previously associated with PCa is *MDH2*, encoding malate dehydrogenase 2. *MDH2* is involved in lipid synthesis and glutamine metabolism in the Krebs cycle. Several proteomics and genomics studies have demonstrated that *MDH2* is a potential biomarker for prognosis and diagnosis of PCa [282–284]. *MDH2* is significantly overexpressed in CRPC [285], and PCa patients with *MDH1* overexpression experienced a shorter period of relapse-free survival after chemotherapy and surgery [286]. Due to these findings, anticancer drugs and treatments targeting MDH1 are being developed, including gene silencing which increases docetaxel sensitivity [286] and alternol treatment [287]. Although *IL4I1* has not been exclusively studied in PCa, pan-cancer studies have shown that the gene is highly expressed in various tumours, including PCa tumours, suggesting a role in cancer development [288, 289]. A study by mention Author(s) [288] has revealed that patients with increased *IL4I1* expression are potentially resistant to treatment with docetaxel [21]. This includes a variant in the interleukin-4 inducible 1 (*IL4I1*) gene, an L-amino acid oxidase that hinders T-cell proliferation [290] and promotes accumulation of palmitate and triglyceride [291]. Similarly, the *ADH1C* gene has not been extensively studied in PCa, especially compared to other alcohol dehydrogenases ADHs such as *ADH1B* and *ADH7* however it has been found to be expressed in PCa tumours at low levels [292].

Interestingly, a variant of the most altered gene in the somatic tumours, *FASN* rs45507397, was identified in the case-control study as one of the variants most associated with PCa (figure 11). This variant was exclusive to African-derived tumours, further highlighting the potential implications of FAM in Southern African PCa. Due to its significant role in long chain saturated fatty acid synthesis, FASN is currently a therapeutic target in multiple ongoing drug trials [293]. *FASN* is already a well-known PCa prognostic marker as several studies have proven it to overexpressed in PCa tumours [204, 294, 295].

In the European cohort, three novel PDVs in *HAO2, REEP6, and METAP1* were identified. The association between these genes and PCa is understudied. Hydroxyacid oxidase 2 (*HAO2*) has been extensively studied as a biomarker for prognosis of HCC because of its downregulation in HCC tumours [296, 297]. The role of *HAO2* in PCa has not been evaluated except for one study which found *HAO2* to be highly expressed in a high-risk PCa group [298]. In one study, receptor accessory protein 6 (*REEP6)* was upregulated in mouse PCa models [299] and in another *REEP6* was predicted to regulate expression of *CXCR4* [300], a chemokine receptor whose overexpression in PCa is linked to prostate tumour metastasis [301]. *REEP6* regulates the cAMP-PKA signalling pathway in adipocytes [302]. The other PDV was found in *METAP1*. Several anticancer drugs that target methionine aminopeptidase 1 (*METAP1*) are continuously being developed and tested, including fumagillin [303], ovalicin [304], flurbiprofen-thioester [305] and tezosentan [306]. *METAP1* has several functions including angiogenesis, protein maturation, and regulating lipid metabolism[305]. The loss of function variant in *FMO1* from the European-derived cohort is another novel PDV found in this study. *FMO1*, flavin containing dimethylaniline monooxygenase 1, metabolizes a variety of xenobiotics [307] and has been reported to be upregulated in mice after castration [308]. The identification of these PDVs in European-derived tumours suggests that they may have a role to play in PCa development and progression.

In this study, the sample size and number of SNPs analysed were relatively small, particularly for the tumour–normal WGS cohort, which reduced the statistical power to detect associations at the conventional GWAS significance threshold of 5×10^{-8}. A more relaxed threshold of 0.05 was therefore used; however, no correction for multiple testing was applied because the small dataset made stringent corrections overly conservative, increasing the risk of missing true associations. This approach increases the risk of false positives, especially for common SNP associations. The limited representation of African genomes in public databases further constrained rare variant interpretation, potentially leading to misclassification. Other limitations include lack of clinical outcome data for the African cohort, and possible biases such as selection bias in the SAPCS cohort or sequencing artefacts. Together, these constraints reduce the robustness, interpretability, and generalizability of the findings. As such, results should be interpreted with caution, and future validation in larger, well-annotated African cohorts is critical to confirm these associations.

CHAPTER 6: CONCLUSION AND FUTURE WORK

Prostate cancer disproportionately affects men of Southern African ancestry, who often experience more aggressive disease and poorer outcomes. This study investigated the FAM pathway's role in PCa by analyzing common, rare, and somatic variants in FAM genes, aiming to identify ancestry-specific genetic factors contributing to aggressive PCa. The study identified nine common germline variants significantly associated with prostate cancer, and eight linked specifically to high-risk disease. These variants may serve as the basis for developing polygenic risk scores tailored to Southern African men, offering potential improvements in early detection and risk stratification. In addition to common variants, fifteen rare pathogenic variants were detected, including novel damaging variants in *PDHB*, *UGDH*, *ENO3*, and *ACADVL* identified in the South African cohort. The observation that most individuals with these rare damaging variants presented with high-risk PCa highlights their potential role in disease severity and reinforces the importance of rare variant screening in African populations. Further interrogation of variants of uncertain significance uncovered four potentially oncogenic variants in *FH*, *SDHD*, *LDHA*, and *NBN*, highlighting the potential need for reclassification of these variants. Additionally, the tumour-derived *FASN* variant rs45507397, which ranked among the top associated variants in the case-control analysis, may have functional significance in African prostate tumours due to *FASN*'s known role in lipid biosynthesis and tumour progression.

This study confirms the central hypothesis that men of Southern African ancestry harbour significant deleterious alterations in the FAM pathway, evidenced by elevated tumour mutational burden and a greater number of damaging variants across common germline, rare pathogenic, and somatic mutation classes. By integrating these variant types, the work reveals the FAM pathway as a recurrently disrupted molecular driver of prostate cancer aggressiveness in this population. Importantly, this represents the first comprehensive FAM-focused genetic study in Southern African men, filling a critical gap in African prostate cancer genomics. The findings have significant clinical implications for improving risk prediction, guiding biomarker development, and informing targeted therapies tailored to African populations, thereby advancing health equity and precision oncology.

Future work includes associating physical features such as body mass index, resting heart rate, blood pressure, and cholesterol with PCa risk to better understand how systemic metabolic health influences cancer development and progression. These factors may serve as accessible clinical biomarkers or targets for prevention strategies. Additionally, a a larger multi-country African cohort from Namibia, Kenya, South Africa, and Nigeria will be included and the gene list will be expanded to increase statistical power and capture a more comprehensive view of genetic contributors to PCa, particularly in FAM. Serum-based lipidomic analysis will also be performed, which could uncover metabolic signatures or dysregulated lipid pathways associated with tumour biology, offering potential for novel diagnostic or therapeutic insights. This future work will be funded by the HEROIC Prostate Cancer Precision Health (PCaPH) Africa1K Project. Consortium efforts like H3Africa and PCaPH are invaluable for advancing genomic research in Africa by promoting data sharing, capacity building, and increased statistical power to better understand genetic diversity and disease susceptibility.

REFERENCES

1 Samtal, C., El Jaddaoui, I., Hamdi, S., Bouguenouch, L., Ouldim, K., Nejjari, C., et al. (2022) Review of prostate cancer genomic studies in Africa. *Front. Genet.*, Frontiers **13** https://doi.org/10.3389/fgene.2022.911101

2 Tan, S.-H., Petrovics, G. and Srivastava, S. (2018) Prostate Cancer Genomics: Recent Advances and the Prevailing Underrepresentation from Racial and Ethnic Minorities. *Int J Mol Sci* **19**, 1255 https://doi.org/10.3390/ijms19041255

3 Galbraith, L., Leung, H. Y. and Ahmad, I. (2018) Lipid pathway deregulation in advanced prostate cancer. *Pharmacological Research* **131**, 177–184 https://doi.org/10.1016/j.phrs.2018.02.022

4 Koundouros, N. and Poulogiannis, G. (2020) Reprogramming of fatty acid metabolism in cancer. *Br J Cancer*, Nature Publishing Group **122**, 4–22 https://doi.org/10.1038/s41416-019-0650-z

5 Caiazza, C., D'Agostino, M., Passaro, F., Faicchia, D., Mallardo, M., Paladino, S., et al. (2019) Effects of Long-Term Citrate Treatment in the PC3 Prostate Cancer Cell Line. *International Journal of Molecular Sciences*, Multidisciplinary Digital Publishing Institute **20**, 2613 https://doi.org/10.3390/ijms20112613

6 Hryniewicz-Jankowska, A., Augoff, K. and Sikorski, A. F. (2019) The role of cholesterol and cholesterol-driven membrane raft domains in prostate cancer. *Exp Biol Med (Maywood)* **244**, 1053–1061 https://doi.org/10.1177/1535370219870771

7 Wasinger, G., Oszwald, A., Shariat, S. F. and Compérat, E. (2022) Histological patterns, subtypes and aspects of prostate cancer: different aspects, different outcomes. *Current Opinion in Urology* **32**, 643 https://doi.org/10.1097/MOU.0000000000001038

8 Alvarado-Cabrero, I., Chaux, A., Muneer, A. and Tamboli, P. (2022) WHO Classification of Tumours Editorial Board. Urinary and male genital tumours. 5th ed.

9 Ilic, D., Djulbegovic, M., Jung, J. H., Hwang, E. C., Zhou, Q., Cleves, A., et al. (2018) Prostate cancer screening with prostate-specific antigen (PSA) test: a systematic review and meta-analysis. *BMJ* **362**, k3519 https://doi.org/10.1136/bmj.k3519

10 Georgiou, L. A. and Scarbrough, B. E. (2024) PSA screening for prostate cancer in the United States: 30 years of controversy. *J Public Health Policy* **45**, 552–561 https://doi.org/10.1057/s41271-024-00502-4

11 Cornford, P., van den Bergh, R. C. N., Briers, E., Van den Broeck, T., Brunckhorst, O., Darraugh, J., et al. (2024) EAU-EANM-ESTRO-ESUR-ISUP-SIOG Guidelines on Prostate Cancer—2024 Update. Part I: Screening, Diagnosis, and Local Treatment with Curative Intent. *European Urology* **86**, 148–163 https://doi.org/10.1016/j.eururo.2024.03.027

12 Albertsen, P. C. (2023) PSA testing, cancer treatment, and prostate cancer mortality reduction: What is the mechanism? *Urol Oncol* **41**, 78–81 https://doi.org/10.1016/j.urolonc.2021.08.010

13 Sekhoacha, M., Riet, K., Motloung, P., Gumenku, L., Adegoke, A. and Mashele, S. (2022) Prostate Cancer Review: Genetics, Diagnosis, Treatment Options, and Alternative Approaches. *Molecules*, Multidisciplinary Digital Publishing Institute **27**, 5730 https://doi.org/10.3390/molecules27175730

14 Godtman, R. A., Kollberg, K. S., Pihl, C.-G., Månsson, M. and Hugosson, J. (2022) The Association Between Age, Prostate Cancer Risk, and Higher Gleason Score in a Long-

term Screening Program: Results from the Göteborg-1 Prostate Cancer Screening Trial. *European Urology* **82**, 311–317 https://doi.org/10.1016/j.eururo.2022.01.018
15 Tilki, D., van den Bergh, R. C. N., Briers, E., Van den Broeck, T., Brunckhorst, O., Darraugh, J., et al. (2024) EAU-EANM-ESTRO-ESUR-ISUP-SIOG Guidelines on Prostate Cancer. Part II-2024 Update: Treatment of Relapsing and Metastatic Prostate Cancer. *Eur Urol* **86**, 164–182 https://doi.org/10.1016/j.eururo.2024.04.010
16 Deweerdt, S. (2015) Prognosis: Proportionate response. *Nature*, Nature Publishing Group **528**, S124–S125 https://doi.org/10.1038/528S124a
17 Efesoy, O., Çayan, S., Aşcı, R., Orhan, İ. and Yaman, Ö. (2021) Hematospermia is rarely related to genitourinary cancer: lessons learned from 15 years of experience with 342 cases. *Int J Impot Res* **33**, 627–633 https://doi.org/10.1038/s41443-020-0330-9
18 Merriel, S. W. D., Funston, G. and Hamilton, W. (2018) Prostate Cancer in Primary Care. *Adv Ther* **35**, 1285–1294 https://doi.org/10.1007/s12325-018-0766-1
19 Litwin, M. S. and Tan, H.-J. (2017) The Diagnosis and Treatment of Prostate Cancer: A Review. *JAMA* **317**, 2532–2542 https://doi.org/10.1001/jama.2017.7248
20 Makau-Barasa, L. K., Manirakiza, A., Carvalho, A. L. and Rebbeck, T. R. (2022) Prostate Cancer Screening, Diagnostic, Treatment Procedures and Costs in Sub-Saharan Africa: A Situational Analysis. *Cancer Control* **29**, 10732748221084932 https://doi.org/10.1177/10732748221084932
21 Xu, Y., Pachnikova, G., Przybilla, D., Schäfer, R., Cui, Y., Zhou, D., et al. (2022) Evaluation of JQ1 Combined With Docetaxel for the Treatment of Prostate Cancer Cells in 2D- and 3D-Culture Systems. *Front Pharmacol* **13**, 839620 https://doi.org/10.3389/fphar.2022.839620
22 Wang, B.-R., Chen, Y.-A., Kao, W.-H., Lai, C.-H., Lin, H. and Hsieh, J.-T. (2022) Developing New Treatment Options for Castration-Resistant Prostate Cancer and Recurrent Disease. *Biomedicines*, Multidisciplinary Digital Publishing Institute **10**, 1872 https://doi.org/10.3390/biomedicines10081872
23 Zhong, S., Jeong, J.-H., Huang, C., Chen, X., Dickinson, S. I., Dhillon, J., et al. (2021) Targeting INMT and interrupting its methylation pathway for the treatment of castration resistant prostate cancer. *J Exp Clin Cancer Res* **40**, 307 https://doi.org/10.1186/s13046-021-02109-z
24 Gandaglia, G., Leni, R., Bray, F., Fleshner, N., Freedland, S. J., Kibel, A., et al. (2021) Epidemiology and Prevention of Prostate Cancer. *Eur Urol Oncol* **4**, 877–892 https://doi.org/10.1016/j.euo.2021.09.006
25 Filho, A. M., Laversanne, M., Ferlay, J., Colombet, M., Piñeros, M., Znaor, A., et al. (2025) The GLOBOCAN 2022 cancer estimates: Data sources, methods, and a snapshot of the cancer burden worldwide. *International Journal of Cancer* **156**, 1336–1346 https://doi.org/10.1002/ijc.35278
26 Rawla, P. (2019) Epidemiology of Prostate Cancer. *World J Oncol* **10**, 63–89 https://doi.org/10.14740/wjon1191
27 Wang, L., Lu, B., He, M., Wang, Y., Wang, Z. and Du, L. (2022) Prostate Cancer Incidence and Mortality: Global Status and Temporal Trends in 89 Countries From 2000 to 2019. *Frontiers in Public Health* **10**
28 Mahal, B. A., Gerke, T., Awasthi, S., Soule, H. R., Simons, J. W., Miyahira, A., et al. (2022) Prostate Cancer Racial Disparities: A Systematic Review by the Prostate Cancer Foundation Panel. *European Urology Oncology* **5**, 18–29 https://doi.org/10.1016/j.euo.2021.07.006
29 International Agency for Research on Cancer. (2022) GLOBOCAN 2022: Global cancer statistics, World Health Organization

30 Bergengren, O., Pekala, K. R., Matsoukas, K., Fainberg, J., Mungovan, S. F., Bratt, O., et al. (2023) 2022 Update on Prostate Cancer Epidemiology and Risk Factors-A Systematic Review. *Eur Urol* **84**, 191–206 https://doi.org/10.1016/j.eururo.2023.04.021
31 Zhai, Z., Zheng, Y., Li, N., Deng, Y., Zhou, L., Tian, T., et al. (2020) Incidence and disease burden of prostate cancer from 1990 to 2017: Results from the Global Burden of Disease Study 2017. *Cancer* **126**, 1969–1978 https://doi.org/10.1002/cncr.32733
32 Abeshouse, A., Ahn, J., Akbani, R., Ally, A., Amin, S., Andry, C. D., et al. (2015) The Molecular Taxonomy of Primary Prostate Cancer. *Cell* **163**, 1011–1025 https://doi.org/10.1016/j.cell.2015.10.025
33 Ferlay, J., Lam, F., Laversanne, M., Colombet, M., Mery, L., Piñeros, M., et al. (2022) GLOBOCAN Botswana Factsheet 2022
34 Bray, F., Laversanne, M., Sung, H., Ferlay, J., Siegel, R. L., Soerjomataram, I., et al. (2024) Global cancer statistics 2022: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin* **74**, 229–263 https://doi.org/10.3322/caac.21834
35 Swart, N. C., Lebelonyane, R., Gutterman, E. M., Legwaila, M., Friebel-Klingner, T. M., Antony, R., et al. (2024) Expanding Geographical Access to Cancer Care in Botswana: Current Status and Future Prospects. *JCO Glob Oncol*, Wolters Kluwer e2400196 https://doi.org/10.1200/GO.24.00196
36 Makau-Barasa, L. K., Manirakiza, A., Carvalho, A. L. and Rebbeck, T. R. (2022) Prostate Cancer Screening, Diagnostic, Treatment Procedures and Costs in Sub-Saharan Africa: A Situational Analysis. *Cancer Control* **29**, 10732748221084932 https://doi.org/10.1177/10732748221084932
37 Anakwenze, C., Bhatia, R., Rate, W., Bakwenabatsile, L., Ngoni, K., Rayne, S., et al. (2018) Factors Related to Advanced Stage of Cancer Presentation in Botswana. *JGO*, Wolters Kluwer 1–9 https://doi.org/10.1200/JGO.18.00129
38 Breul, J. and Wiegel, T. (2016) Strahlentherapeutisches Verfahren vs. radikale Operation. *Urologe* **55**, 311–312 https://doi.org/10.1007/s00120-016-0043-3
39 Di Bello, F., Scheipner, L., Baudo, A., de Angelis, M., Jannello, L. M. I., Siech, C., et al. (2025) Cancer-specific mortality after radical prostatectomy versus radiotherapy in incidental prostate cancer. *Urol Oncol* S1078-1439(24)01057–3 https://doi.org/10.1016/j.urolonc.2024.12.278
40 Esengin, O. (2023, August 14) Botswana Opens its First Public Radiotherapy Centre, With IAEA Support. *International Atomic Energy Agency*, Text, IAEA https://www.iaea.org/newscenter/news/botswana-opens-its-first-public-radiotherapy-centre-with-iaea-support
41 Tapela, N. M., Peluso, M. J., Kohler, R. E., Setlhako, I. I., Botebele, K., Gabegwe, K., et al. (2018) A Step Toward Timely Referral and Early Diagnosis of Cancer: Implementation and Impact on Knowledge of a Primary Care-Based Training Program in Botswana. *Front. Oncol.*, Frontiers **8** https://doi.org/10.3389/fonc.2018.00187
42 Moahi, K., Ralefala, T., Nkele, I., Triedman, S., Sohani, A., Musimar, Z., et al. (2022) HIV and Hodgkin Lymphoma Survival: A Prospective Study in Botswana. *JCO Glob Oncol* **8**, e2100163 https://doi.org/10.1200/GO.21.00163
43 Koobotse, M. O., Zachariah, M., Senabye, B., Gobe, I., Kadimo, K., Nthontho, K. C., et al. (2023) Bibliometric analysis of cancer research outputs in Botswana between 2009 and 2021. *J Cancer Policy* **35**, 100405 https://doi.org/10.1016/j.jcpo.2023.100405
44 Ramatlho, P., Rugemalila, M. M., Tawe, L., Pain, D., Choga, O. T., Ndlovu, A. K., et al. (2025) The Role of Immunohistochemistry for AMACR/p504s and p63 in Distinguishing Prostate Cancer from Benign Prostate Tissue Samples in Botswana. *RRU*, Dove Press **17**, 1–10 https://doi.org/10.2147/RRU.S492935

45 Berenguer, C. V., Pereira, F., Câmara, J. S. and Pereira, J. A. M. (2023) Underlying Features of Prostate Cancer-Statistics, Risk Factors, and Emerging Methods for Its Diagnosis. *Curr Oncol* **30**, 2300–2321 https://doi.org/10.3390/curroncol30020178
46 Dickerman, B. and Mucci, L. (2019) Metabolic Factors and Prostate Cancer Risk. *Clin Chem* **65**, 42–44 https://doi.org/10.1373/clinchem.2018.287243
47 Matsushita, M., Fujita, K. and Nonomura, N. (2020) Influence of Diet and Nutrition on Prostate Cancer. *Int J Mol Sci* **21**, 1447 https://doi.org/10.3390/ijms21041447
48 Cirne, F., Kappel, C., Zhou, S., Mukherjee, S. D., Dehghan, M., Petropoulos, J.-A., et al. (2022) Modifiable risk factors for prostate cancer in low- and lower-middle-income countries: a systematic review and meta-analysis. *Prostate Cancer Prostatic Dis* **25**, 453–462 https://doi.org/10.1038/s41391-022-00570-1
49 Gandaglia, G., Leni, R., Bray, F., Fleshner, N., Freedland, S. J., Kibel, A., et al. (2021) Epidemiology and Prevention of Prostate Cancer. *European Urology Oncology* **4**, 877–892 https://doi.org/10.1016/j.euo.2021.09.006
50 Hwang, T., Oh, H., Lee, J. A. and Kim, E. J. (2023) Prostate cancer risk prediction based on clinical factors and prostate-specific antigen. *BMC Urol* **23**, 100 https://doi.org/10.1186/s12894-023-01259-w
51 Bergengren, O., Pekala, K. R., Matsoukas, K., Fainberg, J., Mungovan, S. F., Bratt, O., et al. (2023) 2022 Update on Prostate Cancer Epidemiology and Risk Factors—A Systematic Review. *Eur Urol* **84**, 191–206 https://doi.org/10.1016/j.eururo.2023.04.021
52 Siegel, R. L., Miller, K. D., Fuchs, H. E. and Jemal, A. (2022) Cancer statistics, 2022. *CA: A Cancer Journal for Clinicians* **72**, 7–33 https://doi.org/10.3322/caac.21708
53 Perdana, N. R., Mochtar, C. A., Umbas, R. and Hamid, A. R. A. (2016) The Risk Factors of Prostate Cancer and Its Prevention: A Literature Review. *Acta Med Indones* **48**, 228–238
54 Hussein, S., Satturwar, S. and Van der Kwast, T. (2015) Young-age prostate cancer. *J Clin Pathol* **68**, 511–515 https://doi.org/10.1136/jclinpath-2015-202993
55 Saliev, T., Akhmad, N., Altynbekova, S., Nogaeva, M., Tazhieva, A. and Dushimova, Z. (2025) Role of ethnic and genetic factors in the development of prostate cancer (Review). *World Academy of Sciences Journal*, Spandidos Publications **7**, 1–14 https://doi.org/10.3892/wasj.2024.301
56 Rawla, P. (2019) Epidemiology of Prostate Cancer. *World J Oncol* **10**, 63–89 https://doi.org/10.14740/wjon1191
57 Jain, M. A., Leslie, S. W. and Sapra, A. (2025) Prostate Cancer Screening. In StatPearls, StatPearls Publishing, Treasure Island (FL)
58 US Preventive Services Task Force. (2018) Screening for Prostate Cancer: US Preventive Services Task Force Recommendation Statement. *JAMA* **319**, 1901–1913 https://doi.org/10.1001/jama.2018.3710
59 Catalona, W. J. (2018) Prostate Cancer Screening. *Med Clin North Am* **102**, 199–214 https://doi.org/10.1016/j.mcna.2017.11.001
60 Denijs, F. B., Van Poppel, H., Stenzl, A., Villanueva, T., Vilaseca, J. M., Ungan, M., et al. (2024) PSA testing in primary care: is it time to change our practice? *BMC Primary Care* **25**, 436 https://doi.org/10.1186/s12875-024-02688-8
61 Boyle, H. J., Alibhai, S., Decoster, L., Efstathiou, E., Fizazi, K., Mottet, N., et al. (2019) Updated recommendations of the International Society of Geriatric Oncology on prostate cancer management in older patients. *European Journal of Cancer* **116**, 116–136 https://doi.org/10.1016/j.ejca.2019.04.031
62 Khodabandeh, M. (2024) Prostate Cancer and Quality of Life in the Elderly: A Literature Review. *Translational Research in Urology*, Tehran University of Medical Sciences **6**, 76–83 https://doi.org/10.22034/tru.2024.460561.1184

63 Hall, R., Bancroft, E., Pashayan, N., Kote-Jarai, Z. and Eeles, R. A. (2024) Genetics of prostate cancer: a review of latest evidence. *Journal of Medical Genetics*, BMJ Publishing Group Ltd **61**, 915–926 https://doi.org/10.1136/jmg-2024-109845
64 Vietri, M. T., D'Elia, G., Caliendo, G., Resse, M., Casamassimi, A., Passariello, L., et al. (2021) Hereditary Prostate Cancer: Genes Related, Target Therapy and Prevention. *International Journal of Molecular Sciences*, Multidisciplinary Digital Publishing Institute **22**, 3753 https://doi.org/10.3390/ijms22073753
65 Hall, R., Bancroft, E., Pashayan, N., Kote-Jarai, Z. and Eeles, R. A. (2024) Genetics of prostate cancer: a review of latest evidence. *Journal of Medical Genetics*, BMJ Publishing Group Ltd **61**, 915–926 https://doi.org/10.1136/jmg-2024-109845
66 Barber, L., Gerke, T., Markt, S. C., Peisch, S. F., Wilson, K. M., Ahearn, T., et al. (2018) Family history of breast or prostate cancer and prostate cancer risk. *Clin Cancer Res* **24**, 5910–5917 https://doi.org/10.1158/1078-0432.CCR-18-0370
67 Ji, C., Ge, W., Zhu, C., Shen, F., Yu, Y., Pang, G., et al. (2025) Family history and genetic risk score combined to guide cancer risk stratification: A prospective cohort study. *International Journal of Cancer* **156**, 505–517 https://doi.org/10.1002/ijc.35187
68 Ren, Z.-J., Cao, D.-H., Zhang, Q., Ren, P.-W., Liu, L.-R., Wei, Q., et al. (2019) First-degree family history of breast cancer is associated with prostate cancer risk: a systematic review and meta-analysis. *BMC Cancer* **19**, 871 https://doi.org/10.1186/s12885-019-6055-9
69 Zheng, Q., Ying, Q., Ren, Z., Zhang, Q., Lu, D., Wang, H., et al. (2021) First-degree family history of prostate cancer is associated the risk of breast cancer and ovarian cancer. *Medicine (Baltimore)* **100**, e23816 https://doi.org/10.1097/MD.0000000000023816
70 Hassanin, E., May, P., Aldisi, R., Spier, I., Forstner, A. J., Nöthen, M. M., et al. (2022) Breast and prostate cancer risk: The interplay of polygenic risk, rare pathogenic germline variants, and family history. *Genet Med* **24**, 576–585 https://doi.org/10.1016/j.gim.2021.11.009
71 Ren, Z.-J., Cao, D.-H., Zhang, Q., Ren, P.-W., Liu, L.-R., Wei, Q., et al. (2019) First-degree family history of breast cancer is associated with prostate cancer risk: a systematic review and meta-analysis. *BMC Cancer* **19**, 871 https://doi.org/10.1186/s12885-019-6055-9
72 Barber, L., Gerke, T., Markt, S. C., Peisch, S. F., Wilson, K. M., Ahearn, T., et al. (2018) Family history of breast or prostate cancer and prostate cancer risk. *Clin Cancer Res* **24**, 5910–5917 https://doi.org/10.1158/1078-0432.CCR-18-0370
73 Rebbeck, T. R. (2018) Prostate Cancer Disparities by Race and Ethnicity: From Nucleotide to Neighborhood. *Cold Spring Harb Perspect Med* **8**, a030387 https://doi.org/10.1101/cshperspect.a030387
74 Fink, B. A., Son, Y., Toumazos, K. C., DeMario, V. K., Okoye, F., Moriarty, R., et al. (2025) A Retrospective Review of the Impact of Racial Disparities and Outcomes on Urinary Anastomotic Leak in Radical Prostatectomy in a National Surgical Registry. *Cureus*, Cureus **17** https://doi.org/10.7759/cureus.80836
75 McHugh, J., Saunders, E. J., Dadaev, T., McGrowder, E., Bancroft, E., Kote-Jarai, Z., et al. (2022) Prostate cancer risk in men of differing genetic ancestry and approaches to disease screening and management in these groups. *Br J Cancer* **126**, 1366–1373 https://doi.org/10.1038/s41416-021-01669-3
76 Biasatti, A., Pavan, N. and Autorino, R. (2025) Androgen deprivation therapy, cardiovascular risk, and mortality in black prostate cancer patients: challenging established beliefs? *Prostate Cancer Prostatic Dis*, Nature Publishing Group 1–2 https://doi.org/10.1038/s41391-025-00966-9

77 McHugh, J., Saunders, E. J., Dadaev, T., McGrowder, E., Bancroft, E., Kote-Jarai, Z., et al. (2022) Prostate cancer risk in men of differing genetic ancestry and approaches to disease screening and management in these groups. *Br J Cancer* **126**, 1366–1373 https://doi.org/10.1038/s41416-021-01669-3
78 Zhang, G., Wang, Z., Bavarva, J., Kuhns, K. J., Guo, J., Ledet, E. M., et al. (2022) A Recurrent ADPRHL1 Germline Mutation Activates PARP1 and Confers Prostate Cancer Risk in African American Families. *Mol Cancer Res* **20**, 1776–1784 https://doi.org/10.1158/1541-7786.MCR-21-0874
79 Na, R., Wei, J., Sample, C. J., Gielzak, M., Choi, S., Cooney, K. A., et al. (2022) The HOXB13 variant X285K is associated with clinical significance and early age at diagnosis in African American prostate cancer patients. *Br J Cancer* **126**, 791–796 https://doi.org/10.1038/s41416-021-01622-4
80 Petersen, D. C., Jaratlerdsiri, W., van Wyk, A., Chan, E. K. F., Fernandez, P., Lyons, R. J., et al. (2019) African KhoeSan ancestry linked to high-risk prostate cancer. *BMC Med Genomics* **12**, 82 https://doi.org/10.1186/s12920-019-0537-0
81 Mucci, L. A., Hjelmborg, J. B., Harris, J. R., Czene, K., Havelick, D. J., Scheike, T., et al. (2016) Familial Risk and Heritability of Cancer Among Twins in Nordic Countries. *JAMA* **315**, 68–76 https://doi.org/10.1001/jama.2015.17703
82 Lambert, S. A., Abraham, G. and Inouye, M. (2019) Towards clinical utility of polygenic risk scores. *Hum Mol Genet* **28**, R133–R142 https://doi.org/10.1093/hmg/ddz187
83 Andreassen, O. A., Zuber, V., Thompson, W. K., Schork, A. J., Bettella, F., Djurovic, S., et al. (2014) Shared common variants in prostate cancer and blood lipids. *Int J Epidemiol* **43**, 1205–1214 https://doi.org/10.1093/ije/dyu090
84 Eeles, R., Al Olama, A. A., Berndt, S., Wiklund, F., Conti, D. V., Ahmed, M., et al. (2017) Prostate cancer meta-analysis from more than 145,000 men to identify 65 novel prostate cancer susceptibility loci. *JCO*, Wolters Kluwer **35**, 1–1 https://doi.org/10.1200/JCO.2017.35.6_suppl.1
85 Janivara, R., Chen, W. C., Hazra, U., Baichoo, S., Agalliu, I., Kachambwa, P., et al. (2024) Heterogeneous genetic architectures of prostate cancer susceptibility in sub-Saharan Africa. *Nat Genet*, Nature Publishing Group **56**, 2093–2103 https://doi.org/10.1038/s41588-024-01931-3
86 Wang, A., Shen, J., Rodriguez, A. A., Saunders, E. J., Chen, F., Janivara, R., et al. (2023) Characterizing prostate cancer risk through multi-ancestry genome-wide discovery of 187 novel risk variants. *Nat Genet*, Nature Publishing Group **55**, 2065–2074 https://doi.org/10.1038/s41588-023-01534-4
87 Houlahan, K. E., Livingstone, J., Fox, N. S., Kurganovs, N., Zhu, H., Sietsma Penington, J., et al. (2023) A polygenic two-hit hypothesis for prostate cancer. *J Natl Cancer Inst* **115**, 468–472 https://doi.org/10.1093/jnci/djad001
88 Saunders, E. J., Kote-Jarai, Z. and Eeles, R. A. (2021) Identification of Germline Genetic Variants that Increase Prostate Cancer Risk and Influence Development of Aggressive Disease. *Cancers (Basel)* **13**, 760 https://doi.org/10.3390/cancers13040760
89 Conti, D. V., Darst, B. F., Moss, L. C., Saunders, E. J., Sheng, X., Chou, A., et al. (2021) Trans-ancestry genome-wide association meta-analysis of prostate cancer identifies new susceptibility loci and informs genetic risk prediction. *Nat Genet*, United States **53**, 65–75 https://doi.org/10.1038/s41588-020-00748-0
90 Nguyen-Dumont, T., MacInnis, R. J., Steen, J. A., Theys, D., Tsimiklis, H., Hammet, F., et al. (2020) Rare germline genetic variants and risk of aggressive prostate cancer. *International Journal of Cancer* **147**, 2142–2149 https://doi.org/10.1002/ijc.33024
91 Beebe-Dimmer, J. L., Hathcock, M., Yee, C., Okoth, L. A., Ewing, C. M., Isaacs, W. B., et al. (2015) The HOXB13 G84E Mutation Is Associated with an Increased Risk for

Prostate Cancer and Other Malignancies. *Cancer Epidemiol Biomarkers Prev* **24**, 1366–1372 https://doi.org/10.1158/1055-9965.EPI-15-0247
92 Pritchard, C. C., Mateo, J., Walsh, M. F., De Sarkar, N., Abida, W., Beltran, H., et al. (2016) Inherited DNA-Repair Gene Mutations in Men with Metastatic Prostate Cancer. *N Engl J Med* **375**, 443–453 https://doi.org/10.1056/NEJMoa1603144
93 Mijuskovic, M., Saunders, E. J., Leongamornlert, D. A., Wakerell, S., Whitmore, I., Dadaev, T., et al. (2019) Correction: Rare germline variants in DNA repair genes and the angiogenesis pathway predispose prostate cancer patients to develop metastatic disease. *Br J Cancer* **120**, 867 https://doi.org/10.1038/s41416-019-0419-4
94 Gheybi, K., Jiang, J., Mutambirwa, S. B. A., Soh, P. X. Y., Kote-Jarai, Z., Jaratlerdsiri, W., et al. (2023) Evaluating Germline Testing Panels in Southern African Males With Advanced Prostate Cancer. *J Natl Compr Canc Netw* **21**, 289-296.e3 https://doi.org/10.6004/jnccn.2022.7097
95 Hayes, V., Gong, T., Jiang, J., Bornman, R., Gheybi, K., Stricker, P., et al. (2024) Rare pathogenic structural variants show potential to enhance prostate cancer germline testing for African men. *Res Sq* rs.3.rs-4531885 https://doi.org/10.21203/rs.3.rs-4531885/v1
96 Wei, J., Beebe-Dimmer, J., Shi, Z., Sample, C., Yan, G., Rifkin, A. S., et al. (2023) Association of rare, recurrent nonsynonymous variants in the germline of prostate cancer patients of African ancestry. *The Prostate* **83**, 454–461 https://doi.org/10.1002/pros.24477
97 White, J. A., Kaninjing, E. T., Adeniji, K. A., Jibrin, P., Obafunwa, J. O., Ogo, C. N., et al. (2024) Whole-exome sequencing of Nigerian benign prostatic hyperplasia reveals increased alterations in apoptotic pathways. *Prostate* **84**, 460–472 https://doi.org/10.1002/pros.24662
98 Wei, J., Beebe-Dimmer, J., Shi, Z., Sample, C., Yan, G., Rifkin, A. S., et al. (2023) Association of rare, recurrent nonsynonymous variants in the germline of prostate cancer patients of African ancestry. *Prostate* **83**, 454–461 https://doi.org/10.1002/pros.24477
99 Darst, B. F., Hughley, R., Pfennig, A., Hazra, U., Fan, C., Wan, P., et al. (2022) A Rare Germline HOXB13 Variant Contributes to Risk of Prostate Cancer in Men of African Ancestry. *Eur Urol* **81**, 458–462 https://doi.org/10.1016/j.eururo.2021.12.023
100 Schaeffer, E. M., Srinivas, S., Adra, N., An, Y., Bitting, R., Chapin, B., et al. (2024) NCCN Guidelines® Insights: Prostate Cancer, Version 3.2024. *J Natl Compr Canc Netw* **22**, 140–150 https://doi.org/10.6004/jnccn.2024.0019
101 Cheng, H. H., Sokolova, A. O., Schaeffer, E. M., Small, E. J. and Higano, C. S. (2019) Germline and Somatic Mutations in Prostate Cancer for the Clinician. *Journal of the National Comprehensive Cancer Network*, National Comprehensive Cancer Network **17**, 515–521 https://doi.org/10.6004/jnccn.2019.7307
102 Cheng, H. H., Sokolova, A. O., Schaeffer, E. M., Small, E. J. and Higano, C. S. (2019) Germline and Somatic Mutations in Prostate Cancer for the Clinician. *Journal of the National Comprehensive Cancer Network*, National Comprehensive Cancer Network **17**, 515–521 https://doi.org/10.6004/jnccn.2019.7307
103 Alvarez-Cubero, M. J., Martinez-Gonzalez, L. J., Robles-Fernandez, I., Martinez-Herrera, J., Garcia-Rodriguez, G., Pascual-Geler, M., et al. (2017) Somatic Mutations in Prostate Cancer: Closer to Personalized Medicine. *Mol Diagn Ther* **21**, 167–178 https://doi.org/10.1007/s40291-016-0248-6
104 Martinez-Gonzalez, L. J., Pascual Geler, M., Robles Fernandez, I., Cozar, J. M., Lorente, J. A. and Alvarez Cubero, M. J. (2018) Improving the genetic signature of prostate cancer, the somatic mutations. *Urol Oncol* **36**, 312.e17-312.e23 https://doi.org/10.1016/j.urolonc.2018.03.012

105 Cotter, K. and Rubin, M. A. (2022) The evolving landscape of prostate cancer somatic mutations. *Prostate* **82 Suppl 1**, S13–S24 https://doi.org/10.1002/pros.24353
106 Melo, C. M., Vidotto, T., Chaves, L. P., Lautert-Dutra, W., Reis, R. B. dos and Squire, J. A. (2021) The Role of Somatic Mutations on the Immune Response of the Tumour Microenvironment in Prostate Cancer. *International Journal of Molecular Sciences*, Multidisciplinary Digital Publishing Institute **22**, 9550 https://doi.org/10.3390/ijms22179550
107 Jang, A., Lanka, S. M., Huang, M., Casado, C. V., Caputo, S. A., Sweeney, P. L., et al. (2023) Comparison of circulating tumour DNA between African American and Caucasian patients with metastatic castrate-resistant prostate cancer post-abiraterone and/or enzalutamide. *Prostate* **83**, 1028–1034 https://doi.org/10.1002/pros.24544
108 Ledet, E. M., Mitsiades, N., Jaeger, E. B., Chao, C. Y. and Sartor, A. O. (2023) CDK12 pathogenic mutations in African American and White patients with prostate cancer. *JCO*, Wolters Kluwer **41**, 38–38 https://doi.org/10.1200/JCO.2023.41.6_suppl.38
109 Antonarakis, E. S., Isaacsson Velho, P., Fu, W., Wang, H., Agarwal, N., Sacristan Santos, V., et al. (2020) CDK12-Altered Prostate Cancer: Clinical Features and Therapeutic Outcomes to Standard Systemic Therapies, Poly (ADP-Ribose) Polymerase Inhibitors, and PD-1 Inhibitors. *JCO Precis Oncol* **4**, 370–381 https://doi.org/10.1200/po.19.00399
110 Fraser, M. and Rouette, A. (2019) Prostate Cancer Genomic Subtypes. *Adv Exp Med Biol* **1210**, 87–110 https://doi.org/10.1007/978-3-030-32656-2_5
111 Chalker, C., Chun, B. and Sokolova, A. O. (2024) Germline and somatic mutations in prostate cancer: Implications for treatment. *Current Problems in Cancer* **50**, 101101 https://doi.org/10.1016/j.currproblcancer.2024.101101
112 Kämpjärvi, K., Kim, N. H., Keskitalo, S., Clark, A. D., von Nandelstadh, P., Turunen, M., et al. (2016) Somatic MED12 mutations in prostate cancer and uterine leiomyomas promote tumourigenesis through distinct mechanisms. *Prostate* **76**, 22–31 https://doi.org/10.1002/pros.23092
113 Cotter, K. and Rubin, M. A. (2022) The evolving landscape of prostate cancer somatic mutations. *Prostate* **82**, S13–S24 https://doi.org/10.1002/pros.24353
114 Broderick, J. M. (2023) FDA grants full approval to pembrolizumab for MSI-H/dMMR solid tumours, including genitourinary cancers, MJH Life Sciences **51**
115 Cheng, H. H., Sokolova, A. O., Schaeffer, E. M., Small, E. J. and Higano, C. S. (2019) Germline and Somatic Mutations in Prostate Cancer for the Clinician. *Journal of the National Comprehensive Cancer Network*, National Comprehensive Cancer Network **17**, 515–521 https://doi.org/10.6004/jnccn.2019.7307
116 Sokolova Ao, Schaeffer Em, Ej, S. and Cs, H. (2019) Germline and Somatic Mutations in Prostate Cancer for the Clinician. *Journal of the National Comprehensive Cancer Network : JNCCN*, J Natl Compr Canc Netw **17** https://doi.org/10.6004/jnccn.2019.7307
117 Deluce, J. E., Cardenas, L., Lalani, A.-K., Maleki Vareki, S. and Fernandes, R. (2022) Emerging Biomarker-Guided Therapies in Prostate Cancer. *Current Oncology*, Multidisciplinary Digital Publishing Institute **29**, 5054–5076 https://doi.org/10.3390/curroncol29070400
118 J, Y., Ke, H., Sg, R., S, L., G, B., Y, Y., et al. (2022) Prostate Cancer Transcriptomic Regulation by the Interplay of Germline Risk Alleles, Somatic Mutations, and 3D Genomic Architecture. *Cancer discovery*, Cancer Discov **12** https://doi.org/10.1158/2159-8290.CD-22-0027
119 Stoykova, G. E. and Schlaepfer, I. R. (2019) Lipid Metabolism and Endocrine Resistance in Prostate Cancer, and New Opportunities for Therapy. *International Journal of Molecular Sciences*, Multidisciplinary Digital Publishing Institute **20**, 2626 https://doi.org/10.3390/ijms20112626

120 Kim, H. and Kim, J. K. (2022) Evidence on Statins, Omega-3, and Prostate Cancer: A Narrative Review. *World J Mens Health* **40**, 412–424 https://doi.org/10.5534/wjmh.210139
121 Aucoin, M., Cooley, K., Knee, C., Fritz, H., Balneaves, L. G., Breau, R., et al. (2017) Fish-Derived Omega-3 Fatty Acids and Prostate Cancer: A Systematic Review. *Integr Cancer Ther* **16**, 32–62 https://doi.org/10.1177/1534735416656052
122 Pelser, C., Mondul, A. M., Hollenbeck, A. R. and Park, Y. (2013) Dietary Fat, Fatty Acids and Risk of Prostate Cancer in the NIH-AARP Diet and Health Study. *Cancer Epidemiol Biomarkers Prev* **22**, 697–707 https://doi.org/10.1158/1055-9965.EPI-12-1196-T
123 Mukherjee, A., Bilecz, A. J. and Lengyel, E. (2022) The adipocyte microenvironment and cancer. *Cancer Metastasis Rev* **41**, 575–587 https://doi.org/10.1007/s10555-022-10059-x
124 Dirat, B., Bochet, L., Dabek, M., Daviaud, D., Dauvillier, S., Majed, B., et al. (2011) Cancer-Associated Adipocytes Exhibit an Activated Phenotype and Contribute to Breast Cancer Invasion. *Cancer Research* **71**, 2455–2465 https://doi.org/10.1158/0008-5472.CAN-10-3323
125 Zhang, Z., Wang, W., Kong, P., Feng, K., Liu, C., Sun, T., et al. (2023) New insights into lipid metabolism and prostate cancer (Review). *Int J Oncol* **62**, 74 https://doi.org/10.3892/ijo.2023.5522
126 Dai, C., Dehm, S. M. and Sharifi, N. (2023) Targeting the Androgen Signaling Axis in Prostate Cancer. *J Clin Oncol* **41**, 4267–4278 https://doi.org/10.1200/JCO.23.00433
127 Hou, Z., Huang, S. and Li, Z. (2021) Androgens in prostate cancer: A tale that never ends. *Cancer Lett* **516**, 1–12 https://doi.org/10.1016/j.canlet.2021.04.010
128 Sena, L. A. and Denmeade, S. R. (2021) Fatty Acid Synthesis in Prostate Cancer: Vulnerability or Epiphenomenon? *Cancer Res* **81**, 4385–4393 https://doi.org/10.1158/0008-5472.CAN-21-1392
129 Li, X., Wu, J. B., Chung, L. W. K. and Huang, W.-C. (2015) Anti-cancer efficacy of SREBP inhibitor, alone or in combination with docetaxel, in prostate cancer habouring p53 mutations. *Oncotarget*, Impact Journals **6**, 41018–41032 https://doi.org/10.18632/oncotarget.5879
130 Pujana-Vaquerizo, M., Bozal-Basterra, L. and Carracedo, A. (2024) Metabolic adaptations in prostate cancer. *Br J Cancer* **131**, 1250–1262 https://doi.org/10.1038/s41416-024-02762-z
131 Pardo, J. C., Ruiz de Porras, V., Gil, J., Font, A., Puig-Domingo, M. and Jordà, M. (2022) Lipid Metabolism and Epigenetics Crosstalk in Prostate Cancer. *Nutrients* **14**, 851 https://doi.org/10.3390/nu14040851
132 Li, X., Wu, J. B., Chung, L. W. K. and Huang, W.-C. (2015) Anti-cancer efficacy of SREBP inhibitor, alone or in combination with docetaxel, in prostate cancer habouring p53 mutations. *Oncotarget*, Impact Journals **6**, 41018–41032 https://doi.org/10.18632/oncotarget.5879
133 Yi, J., Zhu, J., Wu, J., Thompson, C. B. and Jiang, X. (2020) Oncogenic activation of PI3K-AKT-mTOR signaling suppresses ferroptosis via SREBP-mediated lipogenesis. *Proc Natl Acad Sci U S A* **117**, 31189–31197 https://doi.org/10.1073/pnas.2017152117
134 Chen, M., Zhang, J., Sampieri, K., Clohessy, J. G., Mendez, L., Gonzalez-Billalabeitia, E., et al. (2018) An aberrant SREBP-dependent lipogenic program promotes metastatic prostate cancer. *Nat Genet*, Nature Publishing Group **50**, 206–218 https://doi.org/10.1038/s41588-017-0027-2
135 Zhang, Z., Wang, W., Kong, P., Feng, K., Liu, C., Sun, T., et al. (2023) New insights into lipid metabolism and prostate cancer (Review). *Int J Oncol* **62**, 74 https://doi.org/10.3892/ijo.2023.5522

136 Kostecka, L. G., Mendez, S., Li, M., Khare, P., Zhang, C., Le, A., et al. (2024) Cancer cells employ lipid droplets to survive toxic stress. *The Prostate* **84**, 644–655 https://doi.org/10.1002/pros.24680
137 Mitra, R., Le, T. T., Gorjala, P. and Goodman, O. B. (2017) Positive regulation of prostate cancer cell growth by lipid droplet forming and processing enzymes DGAT1 and ABHD5. *BMC Cancer* **17**, 631 https://doi.org/10.1186/s12885-017-3589-6
138 Olzmann, J. A. and Carvalho, P. (2019) Dynamics and functions of lipid droplets. *Nat Rev Mol Cell Biol*, Nature Publishing Group **20**, 137–155 https://doi.org/10.1038/s41580-018-0085-z
139 Brejchova, K., Radner, F. P. W., Balas, L., Paluchova, V., Cajka, T., Chodounska, H., et al. (2021) Distinct roles of adipose triglyceride lipase and hormone-sensitive lipase in the catabolism of triacylglycerol estolides. *Proceedings of the National Academy of Sciences*, Proceedings of the National Academy of Sciences **118**, e2020999118 https://doi.org/10.1073/pnas.2020999118
140 Cerk, I. K., Wechselberger, L. and Oberer, M. (2018) Adipose Triglyceride Lipase Regulation: An Overview. *Curr Protein Pept Sci* **19**, 221–233 https://doi.org/10.2174/1389203718666170918160110
141 Bolsoni-Lopes, A. and Alonso-Vale, M. I. C. (2015) Lipolysis and lipases in white adipose tissue – An update. *Arch. Endocrinol. Metab.*, Sociedade Brasileira de Endocrinologia e Metabologia **59**, 335–342 https://doi.org/10.1590/2359-3997000000067
142 Edwards, M. and Mohiuddin, S. S. (2025) Biochemistry, Lipolysis. In StatPearls, StatPearls Publishing, Treasure Island (FL)
143 Cai, C., Chen, S., Ng, P., Bubley, G. J., Nelson, P. S., Mostaghel, E. A., et al. (2011) Intratumoural De Novo Steroid Synthesis Activates Androgen Receptor in Castration-Resistant Prostate Cancer and Is Upregulated by Treatment with CYP17A1 Inhibitors. *Cancer Research* **71**, 6503–6513 https://doi.org/10.1158/0008-5472.CAN-11-0532
144 Nomura, D. K., Lombardi, D. P., Chang, J. W., Niessen, S., Ward, A. M., Long, J. Z., et al. (2011) Monoacylglycerol Lipase Exerts Dual Control over Endocannabinoid and Fatty Acid Pathways to Support Prostate Cancer. *Chemistry & Biology* **18**, 846–856 https://doi.org/10.1016/j.chembiol.2011.05.009
145 Yu, J., Zhang, M., Li, T., Gao, W., Yang, Z., Wang, K., et al. (2024) Monoacylglycerol lipase blockades the senescence-associated secretory phenotype by interfering with NF-κB activation and promotes docetaxel efficacy in prostate cancer. *Oncogene*, Nature Publishing Group **43**, 2835–2849 https://doi.org/10.1038/s41388-024-03132-y
146 Yang, A. and Mottillo, E. P. (2020) Adipocyte Lipolysis: from molecular mechanisms of regulation to disease and therapeutics. *Biochem J* **477**, 985–1008 https://doi.org/10.1042/BCJ20190468
147 Ma, Y., Temkin, S. M., Hawkridge, A. M., Guo, C., Wang, W., Wang, X.-Y., et al. (2018) Fatty acid oxidation: An emerging facet of metabolic transformation in cancer. *Cancer Lett* **435**, 92–100 https://doi.org/10.1016/j.canlet.2018.08.006
148 Nath, A. and Chan, C. (2016) Genetic alterations in fatty acid transport and metabolism genes are associated with metastatic progression and poor prognosis of human cancers. *Sci Rep*, Nature Publishing Group **6**, 18669 https://doi.org/10.1038/srep18669
149 Hotamisligil, G. S. and Bernlohr, D. A. (2015) Metabolic functions of FABPs—mechanisms and therapeutic implications. *Nat Rev Endocrinol*, Nature Publishing Group **11**, 592–605 https://doi.org/10.1038/nrendo.2015.122
150 Pascual, G., Avgustinova, A., Mejetta, S., Martín, M., Castellanos, A., Attolini, C. S.-O., et al. (2017) Targeting metastasis-initiating cells through the fatty acid receptor CD36. *Nature*, Nature Publishing Group **541**, 41–45 https://doi.org/10.1038/nature20791

151 Pan, J., Fan, Z., Wang, Z., Dai, Q., Xiang, Z., Yuan, F., et al. (2019) CD36 mediates palmitate acid-induced metastasis of gastric cancer via AKT/GSK-3β/β-catenin pathway. *Journal of Experimental & Clinical Cancer Research* **38**, 52 https://doi.org/10.1186/s13046-019-1049-7
152 Sun, Q., Zhang, W., Wang, L., Guo, F., Song, D., Zhang, Q., et al. (2018) Hypermethylated *CD36* gene affected the progression of lung cancer. *Gene* **678**, 395–406 https://doi.org/10.1016/j.gene.2018.06.101
153 Sena, L. A. and Denmeade, S. R. (2021) Fatty Acid Synthesis in Prostate Cancer: Vulnerability or Epiphenomenon? *Cancer Res* **81**, 4385–4393 https://doi.org/10.1158/0008-5472.CAN-21-1392
154 Zhang, Z., Wang, W., Kong, P., Feng, K., Liu, C., Sun, T., et al. (2023) New insights into lipid metabolism and prostate cancer (Review). *Int J Oncol* **62**, 74 https://doi.org/10.3892/ijo.2023.5522
155 Sena, L. A. and Denmeade, S. R. (2021) Fatty Acid Synthesis in Prostate Cancer: Vulnerability or Epiphenomenon? *Cancer Res* **81**, 4385–4393 https://doi.org/10.1158/0008-5472.CAN-21-1392
156 Flaig, T. W., Salzmann-Sullivan, M., Su, L.-J., Zhang, Z., Joshi, M., Gijón, M. A., et al. (2017) Lipid catabolism inhibition sensitizes prostate cancer cells to antiandrogen blockade. *Oncotarget* **8**, 56051–56065 https://doi.org/10.18632/oncotarget.17359
157 Sadeghi, R. N., Karami-Tehrani, F. and Salami, S. (2015) Targeting prostate cancer cell metabolism: impact of hexokinase and CPT-1 enzymes. *Tumour Biol* **36**, 2893–2905 https://doi.org/10.1007/s13277-014-2919-4
158 Ma, Y., Temkin, S. M., Hawkridge, A. M., Guo, C., Wang, W., Wang, X.-Y., et al. (2018) Fatty acid oxidation: An emerging facet of metabolic transformation in cancer. *Cancer Lett* **435**, 92–100 https://doi.org/10.1016/j.canlet.2018.08.006
159 Guin, S., Ashokan, A., Pollack, A. and Dhar, S. (2024) Lipid Metabolism Modulatory Cisplatin Prodrug Sensitizes Resistant Prostate Cancer toward Androgen Deprivation Therapy. *ACS Pharmacol Transl Sci* **7**, 2820–2826 https://doi.org/10.1021/acsptsci.4c00301
160 Uo, T., Sprenger, C. C. and Plymate, S. R. (2020) Androgen Receptor Signaling and Metabolic and Cellular Plasticity During Progression to Castration Resistant Prostate Cancer. *Front Oncol* **10**, 580617 https://doi.org/10.3389/fonc.2020.580617
161 Nassar, Z. D., Mah, C. Y., Dehairs, J., Burvenich, I. J., Irani, S., Centenera, M. M., et al. (2020) Human DECR1 is an androgen-repressed survival factor that regulates PUFA oxidation to protect prostate tumour cells from ferroptosis. *eLife* (Sharifi, N., and Murphy, M. E., eds.), eLife Sciences Publications, Ltd **9**, e54166 https://doi.org/10.7554/eLife.54166
162 Mah, C. Y., Nguyen, A. D. T., Niijima, T., Helm, M., Dehairs, J., Ryan, F. J., et al. (2024) Peroxisomal β-oxidation enzyme, DECR2, regulates lipid metabolism and promotes treatment resistance in advanced prostate cancer. *Br J Cancer* **130**, 741–754 https://doi.org/10.1038/s41416-023-02557-8
163 Klenotic, P. A., Page, R. C., Li, W., Amick, J., Misra, S. and Silverstein, R. L. (2013) Molecular Basis of Antiangiogenic Thrombospondin-1 Type 1 Repeat Domain Interactions With CD36. *Arteriosclerosis, Thrombosis, and Vascular Biology*, American Heart Association **33**, 1655–1662 https://doi.org/10.1161/ATVBAHA.113.301523
164 Mwaikambo, B. R., Sennlaub, F., Ong, H., Chemtob, S. and Hardy, P. (2006) Activation of CD36 Inhibits and Induces Regression of Inflammatory Corneal Neovascularization. *Investigative Ophthalmology & Visual Science* **47**, 4356–4364 https://doi.org/10.1167/iovs.05-1656

165 M. Swamynathan, M., Mathew, G., Aziz, A., Gordon, C., Hillowe, A., Wang, H., et al. (2024) FABP5 Inhibition against PTEN-Mutant Therapy Resistant Prostate Cancer. *Cancers*, Multidisciplinary Digital Publishing Institute **16**, 60 https://doi.org/10.3390/cancers16010060
166 Carbonetti, G., Converso, C., Clement, T., Wang, C., Trotman, L. C., Ojima, I., et al. (2020) Docetaxel/cabazitaxel and fatty acid binding protein 5 inhibitors produce synergistic inhibition of prostate cancer growth. *The Prostate* **80**, 88–98 https://doi.org/10.1002/pros.23921
167 Falchook, G., Infante, J., Arkenau, H.-T., Patel, M. R., Dean, E., Borazanci, E., et al. (2021) First-in-human study of the safety, pharmacokinetics, and pharmacodynamics of first-in-class fatty acid synthase inhibitor TVB-2640 alone and with a taxane in advanced tumours. *eClinicalMedicine*, Elsevier **34** https://doi.org/10.1016/j.eclinm.2021.100797
168 Schmidt, L. J., Ballman, K. V. and Tindall, D. J. (2007) Inhibition of fatty acid synthase activity in prostate cancer cells by dutasteride. *The Prostate* **67**, 1111–1120 https://doi.org/10.1002/pros.20602
169 Rae, C., Fragkoulis, G. I. and Chalmers, A. J. (2020) Cytotoxicity and Radiosensitizing Activity of the Fatty Acid Synthase Inhibitor C75 Is Enhanced by Blocking Fatty Acid Uptake in Prostate Cancer Cells. *Advances in Radiation Oncology*, Elsevier **5**, 994–1005 https://doi.org/10.1016/j.adro.2020.06.022
170 Li, X., Chen, Y.-T., Hu, P. and Huang, W.-C. (2014) Fatostatin Displays High Antitumour Activity in Prostate Cancer by Blocking SREBP-Regulated Metabolic Pathways and Androgen Receptor Signaling. *Molecular Cancer Therapeutics* **13**, 855–866 https://doi.org/10.1158/1535-7163.MCT-13-0797
171 Centenera, M. M., Scott, J. S., Machiels, J., Nassar, Z. D., Miller, D. C., Zinonos, I., et al. (2021) ELOVL5 Is a Critical and Targetable Fatty Acid Elongase in Prostate Cancer. *Cancer Research* **81**, 1704–1718 https://doi.org/10.1158/0008-5472.CAN-20-2511
172 Khan, A. S. and Frigo, D. E. (2017) Regulation, Role and Therapeutic Targeting of AMPK in Prostate Cancer. *Nat Rev Urol* **14**, 164–180 https://doi.org/10.1038/nrurol.2016.272
173 Rae, C. and Mairs, R. J. (2019) AMPK activation by AICAR sensitizes prostate cancer cells to radiotherapy. *Oncotarget* **10**, 749–759 https://doi.org/10.18632/oncotarget.26598
174 Zadra, G., Photopoulos, C., Tyekucheva, S., Heidari, P., Weng, Q. P., Fedele, G., et al. (2014) A novel direct activator of AMPK inhibits prostate cancer growth by blocking lipogenesis. *EMBO Molecular Medicine*, Springer Nature **6**, 519–538 https://doi.org/10.1002/emmm.201302734
175 Xiang, X., Saha, A. K., Wen, R., Ruderman, N. B. and Luo, Z. (2004) AMP-activated protein kinase activators can inhibit the growth of prostate cancer cells by multiple mechanisms. *Biochemical and Biophysical Research Communications* **321**, 161–167 https://doi.org/10.1016/j.bbrc.2004.06.133
176 Soh, P. X. Y., Mmekwa, N., Petersen, D. C., Gheybi, K., van Zyl, S., Jiang, J., et al. (2023) Prostate cancer genetic risk and associated aggressive disease in men of African ancestry. *Nat Commun* **14**, 8037 https://doi.org/10.1038/s41467-023-43726-w
177 Durinck, S., Spellman, P. T., Birney, E. and Huber, W. (2009) Mapping identifiers for the integration of genomic datasets with the R/Bioconductor package biomaRt. *Nat Protoc* **4**, 1184–1191 https://doi.org/10.1038/nprot.2009.97
178 Liberzon, A., Birger, C., Thorvaldsdóttir, H., Ghandi, M., Mesirov, J. P. and Tamayo, P. (2015) The Molecular Signatures Database (MSigDB) hallmark gene set collection. *Cell Syst* **1**, 417–425 https://doi.org/10.1016/j.cels.2015.12.004

179 Chang, C. C., Chow, C. C., Tellier, L. C., Vattikuti, S., Purcell, S. M. and Lee, J. J. (2015) Second-generation PLINK: rising to the challenge of larger and richer datasets. *Gigascience* **4**, 7 https://doi.org/10.1186/s13742-015-0047-8
180 Skol, A. D., Scott, L. J., Abecasis, G. R. and Boehnke, M. (2006) Joint analysis is more efficient than replication-based analysis for two-stage genome-wide association studies. *Nat Genet* **38**, 209–213 https://doi.org/10.1038/ng1706
181 Wickham, H. (2016) ggplot2, Springer International Publishing, Cham https://doi.org/10.1007/978-3-319-24277-4
182 Sim, N.-L., Kumar, P., Hu, J., Henikoff, S., Schneider, G. and Ng, P. C. (2012) SIFT web server: predicting effects of amino acid substitutions on proteins. *Nucleic Acids Res* **40**, W452–W457 https://doi.org/10.1093/nar/gks539
183 Adzhubei, I. A., Schmidt, S., Peshkin, L., Ramensky, V. E., Gerasimova, A., Bork, P., et al. (2010) A method and server for predicting damaging missense mutations. *Nat Methods* **7**, 248–249 https://doi.org/10.1038/nmeth0410-248
184 Landrum, M. J., Chitipiralla, S., Brown, G. R., Chen, C., Gu, B., Hart, J., et al. (2020) ClinVar: improvements to accessing data. *Nucleic Acids Res* **48**, D835–D844 https://doi.org/10.1093/nar/gkz972
185 Li, Q. and Wang, K. (2017) InterVar: Clinical Interpretation of Genetic Variants by the 2015 ACMG-AMP Guidelines. *The American Journal of Human Genetics*, Elsevier **100**, 267–280 https://doi.org/10.1016/j.ajhg.2017.01.004
186 Li, H. and Durbin, R. (2010) Fast and accurate long-read alignment with Burrows-Wheeler transform. *Bioinformatics* **26**, 589–595 https://doi.org/10.1093/bioinformatics/btp698
187 Jaratlerdsiri, W., Jiang, J., Gong, T., Patrick, S. M., Willet, C., Chew, T., et al. (2022) African-specific molecular taxonomy of prostate cancer. *Nature* **609**, 552–559 https://doi.org/10.1038/s41586-022-05154-6
188 Wang, K., Li, M. and Hakonarson, H. ANNOVAR: functional annotation of genetic variants from high-throughput sequencing data
189 Rentzsch, P., Schubach, M., Shendure, J. and Kircher, M. (2021) CADD-Splice-improving genome-wide variant effect prediction using deep learning-derived splice scores. *Genome Med* **13**, 31 https://doi.org/10.1186/s13073-021-00835-9
190 Landrum, M. J., Chitipiralla, S., Brown, G. R., Chen, C., Gu, B., Hart, J., et al. (2020) ClinVar: improvements to accessing data. *Nucleic Acids Research* **48**, D835–D844 https://doi.org/10.1093/nar/gkz972
191 Tamborero, D., Rubio-Perez, C., Deu-Pons, J., Schroeder, M. P., Vivancos, A., Rovira, A., et al. (2018) Cancer Genome Interpreter annotates the biological and clinical relevance of tumour alterations. *Genome Medicine* **10**, 25 https://doi.org/10.1186/s13073-018-0531-8
192 Karczewski, K. J., Francioli, L. C., Tiao, G., Cummings, B. B., Alföldi, J., Wang, Q., et al. (2020) The mutational constraint spectrum quantified from variation in 141,456 humans. *Nature*, Nature Publishing Group **581**, 434–443 https://doi.org/10.1038/s41586-020-2308-7
193 Chen, S., Francioli, L. C., Goodrich, J. K., Collins, R. L., Kanai, M., Wang, Q., et al. (2024) A genomic mutational constraint map using variation in 76,156 human genomes. *Nature*, Nature Publishing Group **625**, 92–100 https://doi.org/10.1038/s41586-023-06045-0
194 Craddock, J., Jiang, J., Patrick, S. M., Mutambirwa, S. B. A., Stricker, P. D., Bornman, M. S. R., et al. (2023) Alterations in the Epigenetic Machinery Associated with Prostate Cancer Health Disparities. *Cancers (Basel)* **15**, 3462 https://doi.org/10.3390/cancers15133462

195 Oscanoa, J., Sivapalan, L., Gadaleta, E., Dayem Ullah, A. Z., Lemoine, N. R. and Chelala, C. (2020) SNPnexus: a web server for functional annotation of human genome sequence variation (2020 update). *Nucleic Acids Res* **48**, W185–W192 https://doi.org/10.1093/nar/gkaa420
196 Mayakonda, A., Lin, D.-C., Assenov, Y., Plass, C. and Koeffler, H. P. (2018) Maftools: efficient and comprehensive analysis of somatic variants in cancer. *Genome Res.* **28**, 1747–1756 https://doi.org/10.1101/gr.239244.118
197 Chen, F., Madduri, R. K., Rodriguez, A. A., Darst, B. F., Chou, A., Sheng, X., et al. (2023) Evidence of Novel Susceptibility Variants for Prostate Cancer and a Multiancestry Polygenic Risk Score Associated with Aggressive Disease in Men of African Ancestry. *European Urology* **84**, 13–21 https://doi.org/10.1016/j.eururo.2023.01.022
198 Wang, A., Shen, J., Rodriguez, A. A., Saunders, E. J., Chen, F., Janivara, R., et al. (2023) Characterizing prostate cancer risk through multi-ancestry genome-wide discovery of 187 novel risk variants. *Nat Genet* **55**, 2065–2074 https://doi.org/10.1038/s41588-023-01534-4
199 Janivara, R., Chen, W. C., Hazra, U., Baichoo, S., Agalliu, I., Kachambwa, P., et al. (2024) Heterogeneous genetic architectures of prostate cancer susceptibility in sub-Saharan Africa. *Nat Genet*, Nature Publishing Group **56**, 2093–2103 https://doi.org/10.1038/s41588-024-01931-3
200 Piffer, D. (2016) Evidence of polygenic selection on human stature inferred from spatial distribution of allele frequencies. *F1000Research*, F1000 Research Limited **4**, 15
201 Escala-Garcia, M. (2020) Hanging in there: exploring the heritabilit of breast cancer sur i al. *BMC Medicine* **18**, 327
202 Verleyen, M., He, Y., Burssens, A., Silva, M. S., Callewaert, B. and Audenaert, E. (2024) A systematic review and cross-database analysis of single nucleotide polymorphisms underlying hip morphology and osteoarthritis reveals shared mechanisms. *Osteoarthritis and Cartilage* https://doi.org/10.1016/j.joca.2024.05.010
203 Blackburn, P. R., Gass, J. M., Vairo, F. P. E., Farnham, K. M., Atwal, H. K., Macklin, S., et al. (2017) Maple syrup urine disease: mechanisms and management. *Appl Clin Genet* **10**, 57–66 https://doi.org/10.2147/TACG.S125962
204 Dairo, O., DePaula Oliveira, L., Schaffer, E., Vidotto, T., Mendes, A. A., Lu, J., et al. (2024) FASN Gene Methylation is Associated with Fatty Acid Synthase Expression and Clinical-genomic Features of Prostate Cancer. *Cancer Res Commun* **4**, 152–163 https://doi.org/10.1158/2767-9764.CRC-23-0248
205 Basavaraj, P., Ruangsai, P., Hsieh, P.-F., Jiang, W.-P., Bau, D.-T., Huang, G.-J., et al. (2022) Alpinumisoflavone Exhibits the Therapeutic Effect on Prostate Cancer Cells by Repressing AR and Co-Targeting FASN- and HMGCR-Mediated Lipid and Cholesterol Biosynthesis. *Life (Basel)* **12**, 1769 https://doi.org/10.3390/life12111769
206 Chuang, H.-Y., Lee, Y.-P., Lin, W.-C., Lin, Y.-H. and Hwang, J.-J. (2019) Fatty Acid Inhibition Sensitizes Androgen-Dependent and -Independent Prostate Cancer to Radiotherapy via FASN/NF-κB Pathway. *Sci Rep* **9**, 13284 https://doi.org/10.1038/s41598-019-49486-2
207 Ambrosio, M. R., Di Serio, C., Danza, G., Rocca, B. J., Ginori, A., Prudovsky, I., et al. (2016) Carbonic anhydrase IX is a marker of hypoxia and correlates with higher Gleason scores and ISUP grading in prostate cancer. *Diagn Pathol* **11**, 45 https://doi.org/10.1186/s13000-016-0495-1
208 Angeli, A., Trallori, E., Carta, F., Di Cesare Mannelli, L., Ghelardini, C. and Supuran, C. T. (2018) Heterocoumarins Are Selective Carbonic Anhydrase IX and XII Inhibitors with Cytotoxic Effects against Cancer Cells Lines. *ACS Med Chem Lett* **9**, 947–951 https://doi.org/10.1021/acsmedchemlett.8b00362

209 Logozzi, M., Capasso, C., Di Raimo, R., Del Prete, S., Mizzoni, D., Falchi, M., et al. (2019) Prostate cancer cells and exosomes in acidic condition show increased carbonic anhydrase IX expression and activity. *J Enzyme Inhib Med Chem* **34**, 272–278 https://doi.org/10.1080/14756366.2018.1538980
210 Logozzi, M., Mizzoni, D., Capasso, C., Del Prete, S., Di Raimo, R., Falchi, M., et al. (2020) Plasmatic exosomes from prostate cancer patients show increased carbonic anhydrase IX expression and activity and low pH. *J Enzyme Inhib Med Chem* **35**, 280–288 https://doi.org/10.1080/14756366.2019.1697249
211 Riemann, A., Güttler, A., Haupt, V., Wichmann, H., Reime, S., Bache, M., et al. (2018) Inhibition of Carbonic Anhydrase IX by Ureidosulfonamide Inhibitor U104 Reduces Prostate Cancer Cell Growth, But Does Not Modulate Daunorubicin or Cisplatin Cytotoxicity. *Oncol Res* **26**, 191–200 https://doi.org/10.3727/096504017X14965111926391
212 Saghafi, T., Taheri, R. A., Parkkila, S. and Zolfaghari Emameh, R. (2019) Phytochemicals as Modulators of Long Non-Coding RNAs and Inhibitors of Cancer-Related Carbonic Anhydrases. *International Journal of Molecular Sciences*, Multidisciplinary Digital Publishing Institute **20**, 2939 https://doi.org/10.3390/ijms20122939
213 Lin, A.-P., Abbas, S., Kim, S.-W., Ortega, M., Bouamar, H., Escobedo, Y., et al. (2015) D2HGDH regulates alpha-ketoglutarate levels and dioxygenase function by modulating IDH2. *Nat Commun* **6**, 7768 https://doi.org/10.1038/ncomms8768
214 Yang, Q., Hao, J., Chi, M., Wang, Y., Li, J., Huang, J., et al. (2022) D2HGDH-mediated D2HG catabolism enhances the anti-tumour activities of CAR-T cells in an immunosuppressive microenvironment. *Mol Ther* **30**, 1188–1200 https://doi.org/10.1016/j.ymthe.2022.01.007
215 Hayashi, C., Takagi, K., Sato, A., Yamaguchi, M., Minemura, H., Miki, Y., et al. (2021) D-2-hydroxyglutarate dehydrogenase in breast carcinoma as a potent prognostic marker associated with proliferation. *Histol Histopathol* **36**, 1053–1062 https://doi.org/10.14670/HH-18-362
216 Han, J., Jackson, D., Holm, J., Turner, K., Ashcraft, P., Wang, X., et al. (2018) Elevated d-2-hydroxyglutarate during colitis drives progression to colorectal cancer. *Proc Natl Acad Sci U S A* **115**, 1057–1062 https://doi.org/10.1073/pnas.1712625115
217 Liu, J., Xu, W., Li, S., Sun, R. and Cheng, W. (2020) Multi-omics analysis of tumour mutational burden combined with prognostic assessment in epithelial ovarian cancer based on TCGA database. *Int J Med Sci* **17**, 3200–3213 https://doi.org/10.7150/ijms.50491
218 Wang, J., Chen, X., Tian, Y., Zhu, G., Qin, Y., Chen, X., et al. (2020) Six-gene signature for predicting survival in patients with head and neck squamous cell carcinoma. *Aging (Albany NY)* **12**, 767–783 https://doi.org/10.18632/aging.102655
219 Qin, F., Song, Z., Chang, M., Song, Y., Frierson, H. and Li, H. (2016) Recurrent cis-SAGe chimeric RNA, D2HGDH-GAL3ST2, in prostate cancer. *Cancer Lett* **380**, 39–46 https://doi.org/10.1016/j.canlet.2016.06.013
220 Kan, S., Hou, Q., Shi, J., Zhang, M., Xu, F., Liu, Z., et al. (2024) EHHADH deficiency regulates pexophagy and accelerates tubulointerstitial injury in diabetic kidney disease. *Cell Death Discov* **10**, 289 https://doi.org/10.1038/s41420-024-02066-4
221 Forst, A.-L., Reichold, M., Kleta, R. and Warth, R. (2021) Distinct Mitochondrial Pathologies Caused by Mutations of the Proximal Tubular Enzymes EHHADH and GATM. *Front Physiol* **12**, 715485 https://doi.org/10.3389/fphys.2021.715485
222 Xie Siyu, Li Miaosheng, JIANG Fengle, Yi Qian, and Yang Wei. (2023) EHHADH 是肝细胞癌脂肪酸代谢通路的关键基因：基于转录组分析. *Nan Fang Yi Ke Da Xue Xue Bao* **43**, 680–693 https://doi.org/10.12122/j.issn.1673-4254.2023.05.02

223 Eckel-Passow, J. E., Lachance, D. H., Decker, P. A., Kollmeyer, T. M., Kosel, M. L., Drucker, K. L., et al. (2022) Inherited genetics of adult diffuse glioma and polygenic risk scores-a review. *Neurooncol Pract* **9**, 259–270 https://doi.org/10.1093/nop/npac017
224 He, J., Zhao, H., Deng, D., Wang, Y., Zhang, X., Zhao, H., et al. (2020) Screening of significant biomarkers related with prognosis of liver cancer by lncRNA-associated ceRNAs analysis. *Journal of Cellular Physiology* **235**, 2464–2477 https://doi.org/10.1002/jcp.29151
225 S, X., M, L., F, J., Q, Y. and W, Y. (2023) [EHHADH is a key gene in fatty acid metabolism pathways in hepatocellular carcinoma: a transcriptomic analysis]. *Nan fang yi ke da xue xue bao = Journal of Southern Medical University*, Nan Fang Yi Ke Da Xue Xue Bao **43** https://doi.org/10.12122/j.issn.1673-4254.2023.05.02
226 Li, J., Zheng, Y., Chen, C., Lin, Z., Pan, J., Liang, Y., et al. (2024) Arginase 2 is a Diagnostic and Prognostic Marker for Prostate Cancer and Is Associated with Metabolism. *Altern Ther Health Med* AT10066
227 Filadi, R., De Mario, A., Audano, M., Romani, P., Pedretti, S., Cardenas, C., et al. (2023) Sustained IP3-linked Ca2+ signaling promotes progression of triple negative breast cancer cells by regulating fatty acid metabolism. *Front Cell Dev Biol* **11**, 1071037 https://doi.org/10.3389/fcell.2023.1071037
228 Jianfeng, W., Yutao, W. and Jianbin, B. (2022) Indolethylamine-N-Methyltransferase Inhibits Proliferation and Promotes Apoptosis of Human Prostate Cancer Cells: A Mechanistic Exploration. *Front Cell Dev Biol* **10**, 805402 https://doi.org/10.3389/fcell.2022.805402
229 Zhang, Y. and Li, Y. (2024) β-hydroxybutyrate inhibits malignant phenotypes of prostate cancer cells through β-hydroxybutyrylation of indoleacetamide-N-methyltransferase. *Cancer Cell Int* **24**, 121 https://doi.org/10.1186/s12935-024-03277-6
230 Yang, H., Ralle, M., Wolfgang, M. J., Dhawan, N., Burkhead, J. L., Rodriguez, S., et al. (2018) Copper-dependent amino oxidase 3 governs selection of metabolic fuels in adipocytes. *PLoS Biol* **16**, e2006519 https://doi.org/10.1371/journal.pbio.2006519
231 Ahmad Zawawi, S. S., Mohd Azram, N. A. S., Sulong, S., Zakaria, A. D., Lee, Y. Y., Che Jalil, N. A., et al. (2023) Identification of AOC3 and LRRC17 as Colonic Fibroblast Activation Markers and Their Potential Roles in Colorectal Cancer Progression. *Asian Pac J Cancer Prev* **24**, 3099–3107 https://doi.org/10.31557/APJCP.2023.24.9.3099
232 Xu, H., Cui, H., Weng, S., Zhang, Y., Wang, L., Xing, Z., et al. (2024) Crosstalk of cell death pathways unveils an autophagy-related gene AOC3 as a critical prognostic marker in colorectal cancer. *Commun Biol* **7**, 296 https://doi.org/10.1038/s42003-024-05980-6
233 Cha, Y. J., Jung, W. H. and Koo, J. S. (2018) Site-specific expression of amine oxidases in breast cancer metastases. *Tumour Biol* **40**, 1010428318776822 https://doi.org/10.1177/1010428318776822
234 Sun, W. Y., Choi, J., Cha, Y. J. and Koo, J. S. (2017) Evaluation of the Expression of Amine Oxidase Proteins in Breast Cancer. *Int J Mol Sci* **18**, 2775 https://doi.org/10.3390/ijms18122775
235 Pan, R., Pan, F., Zeng, Z., Lei, S., Yang, Y., Yang, Y., et al. (2022) A novel immune cell signature for predicting osteosarcoma prognosis and guiding therapy. *Front Immunol* **13**, 1017120 https://doi.org/10.3389/fimmu.2022.1017120
236 Zhu, B., Huang, Z.-C., Deng, H.-H., Dong, Z.-T., Zhao, X.-K., Yang, L.-Y., et al. (2020) [Silencing ERp29 promotes the invasiveness of human PCa cells in vitro: Molecular mechanisms]. *Zhonghua Nan Ke Xue* **26**, 579–587
237 Wu, J., Yang, Y., Gao, S., Jiang, H., Wang, X.-Q., Xiao, Y., et al. (2017) ERp29 inhibits tumourigenicity by suppressing epithelial mesenchymal transition in gastric cancer. *Oncotarget*, Impact Journals **8**, 78757–78766 https://doi.org/10.18632/oncotarget.20225

238 Cheng, K.-C., Kuo, H.-C., Hsieh, M.-C., Huang, C.-Y., Teng, C.-C., Tung, S.-Y., et al. (2021) Identification of Two Novel CIL-102 Upregulations of ERP29 and FUMH to Inhibit the Migration and Invasiveness of Colorectal Cancer Cells by Using the Proteomic Approach. *Biomolecules* **11**, 1280 https://doi.org/10.3390/biom11091280
239 Matejka, K., Stückler, F., Salomon, M., Ensenauer, R., Reischl, E., Hoerburger, L., et al. (2019) Dynamic modelling of an ACADS genotype in fatty acid oxidation - Application of cellular models for the analysis of common genetic variants. *PLoS One* **14**, e0216110 https://doi.org/10.1371/journal.pone.0216110
240 Wu, Q., Yan, T., Chen, Y., Chang, J., Jiang, Y., Zhu, D., et al. (2021) Integrated Analysis of Expression and Prognostic Values of Acyl-CoA Dehydrogenase short-chain in Colorectal Cancer. *Int J Med Sci* **18**, 3631–3643 https://doi.org/10.7150/ijms.63953
241 Tao, L., Zhong, L., Li, Y., Li, D., Xiu, D. and Zhou, J. (2021) Integrated proteomics and phosphoproteomics reveal perturbed regulative pathways in pancreatic ductal adenocarcinoma. *Mol Omics* **17**, 230–240 https://doi.org/10.1039/d0mo00125b
242 Chen, D., Feng, X., Lv, Z., Xu, X., Lu, Y., Wu, W., et al. (2019) ACADS acts as a potential methylation biomarker associated with the proliferation and metastasis of hepatocellular carcinomas. *Aging (Albany NY)* **11**, 8825–8844 https://doi.org/10.18632/aging.102292
243 Chow, P. H., Bowen, J. and Yool, A. J. (2020) Combined Systematic Review and Transcriptomic Analyses of Mammalian Aquaporin Classes 1 to 10 as Biomarkers and Prognostic Indicators in Diverse Cancers. *Cancers (Basel)* **12**, 1911 https://doi.org/10.3390/cancers12071911
244 Kushwaha, P. P., Verma, S. and Gupta, S. (2023) Aquaporins as Prognostic Biomarker in Prostate Cancer. *Cancers (Basel)* **15**, 331 https://doi.org/10.3390/cancers15020331
245 Dai, C., Charlestin, V., Wang, M., Walker, Z. T., Miranda-Vergara, M. C., Facchine, B. A., et al. (2020) Aquaporin-7 Regulates the Response to Cellular Stress in Breast Cancer. *Cancer Research* **80**, 4071–4086 https://doi.org/10.1158/0008-5472.CAN-19-2269
246 Itkonen, H. M., Poulose, N., Walker, S. and Mills, I. G. (2019) CDK9 Inhibition Induces a Metabolic Switch that Renders Prostate Cancer Cells Dependent on Fatty Acid Oxidation. *Neoplasia* **21**, 713–720 https://doi.org/10.1016/j.neo.2019.05.001
247 Iglesias-Gato, D., Wikström, P., Tyanova, S., Lavallee, C., Thysell, E., Carlsson, J., et al. (2016) The Proteome of Primary Prostate Cancer. *Eur Urol* **69**, 942–952 https://doi.org/10.1016/j.eururo.2015.10.053
248 Vieira, F. Q., Cardoso, A. R., Gigliano, D., Carneiro, I., Henrique, R., Jerónimo, C., et al. (2021, August 2) LDHA and CPT2 association with therapy resistance in prostate cancer. | European Journal of Public Health | EBSCOhost https://doi.org/10.1093/eurpub/ckab120.068
249 Neuwirt, H., Bouchal, J., Kharaishvili, G., Ploner, C., Jöhrer, K., Pitterl, F., et al. (2020) Cancer-associated fibroblasts promote prostate tumour growth and progression through upregulation of cholesterol and steroid biosynthesis. *Cell Commun Signal* **18**, 11 https://doi.org/10.1186/s12964-019-0505-5
250 Pouliquen, D. L. (2023, May 24) Comparison of Deeply Versus Mildly Invasive Mesothelioma Proteomes Identifies Potential Links Between Mitochondrial Acadvl Expression and Biomarkers of Aggressiveness - [v1], Preprints https://doi.org/10.20944/preprints202305.1698.v1
251 Liu, S., Lai, J., Feng, Y., Zhuo, Y., Zhang, H., Chen, Y., et al. (2023) Acetyl-CoA carboxylase 1 depletion suppresses de novo fatty acid synthesis and mitochondrial β-oxidation in castration-resistant prostate cancer cells. *Journal of Biological Chemistry*, Elsevier **299** https://doi.org/10.1016/j.jbc.2022.102720

252 Chen, J., Zhang, Z., Ni, J., Sun, J., Ju, F., Wang, Z., et al. (2022) ENO3 promotes colorectal cancer progression by enhancing cell glycolysis. *Med Oncol* **39**, 80 https://doi.org/10.1007/s12032-022-01676-1
253 Cui, H., Guo, D., Zhang, X., Zhu, Y., Wang, Z., Jin, Y., et al. (2021) ENO3 Inhibits Growth and Metastasis of Hepatocellular Carcinoma via Wnt/β-Catenin Signaling Pathway. *Front. Cell Dev. Biol.*, Frontiers **9** https://doi.org/10.3389/fcell.2021.797102
254 Zimmer, B. M., Howell, M. E., Ma, L., Enders, J. R., Lehman, D., Corey, E., et al. (2021) Altered glucuronidation deregulates androgen dependent response profiles and signifies castration resistance in prostate cancer. *Oncotarget* **12**, 1886–1902 https://doi.org/10.18632/oncotarget.28059
255 Huang, D., Casale, G. P., Tian, J., Lele, S. M., Pisarev, V. M., Simpson, M. A., et al. (2010) Udp-glucose dehydrogenase as a novel field-specific candidate biomarker of prostate cancer. *Int J Cancer* **126**, 315–327 https://doi.org/10.1002/ijc.24820
256 Zimmer, B. M., Howell, M. E., Wei, Q., Ma, L., Romsdahl, T., Loughman, E. G., et al. (2016) Loss of exogenous androgen dependence by prostate tumour cells is associated with elevated glucuronidation potential. *Horm Cancer* **7**, 260–271 https://doi.org/10.1007/s12672-016-0268-z
257 Chi, C., Hou, W., Zhang, Y., Chen, J., Shen, Z., Chen, Y., et al. (2023) PDHB-AS suppresses cervical cancer progression and cisplatin resistance via inhibition on Wnt/β-catenin pathway. *Cell Death Dis*, Nature Publishing Group **14**, 1–11 https://doi.org/10.1038/s41419-022-05547-5
258 Wang, H., Yang, Z., He, X., Guo, F., Sun, H., Xu, S., et al. (2023, February 3) Cuproptosis related gene PDHB was identified as a biomarker and its up-regulation inhibited the invasion of renal clear cell carcinoma https://doi.org/10.21203/rs.3.rs-2465586/v1
259 Walsh, N., Cooper, A., Dockery, A. and O'Byrne, J. J. (2024) Variant reclassification and clinical implications. *Journal of Medical Genetics*, BMJ Publishing Group Ltd **61**, 207–211 https://doi.org/10.1136/jmg-2023-109488
260 Schmidt, C., Sciacovelli, M. and Frezza, C. (2020) Fumarate hydratase in cancer: A multifaceted tumour suppressor. *Seminars in Cell & Developmental Biology* **98**, 15–25 https://doi.org/10.1016/j.semcdb.2019.05.002
261 Kwon, O. K., Bang, I. H., Choi, S. Y., Jeon, J. M., Na, A.-Y., Gao, Y., et al. (2023) LDHA Desuccinylase Sirtuin 5 as A Novel Cancer Metastatic Stimulator in Aggressive Prostate Cancer. *Genomics Proteomics Bioinformatics* **21**, 177–189 https://doi.org/10.1016/j.gpb.2022.02.004
262 Tian, L., Zhou, N., Zhao, N., Qiao, M., He, M., Mao, Z., et al. (2024) Low level exposure to BDE-47 facilitates the development of prostate cancer through TOP2A/LDHA/lactylation positive feedback circuit. *Environ Res* **263**, 120094 https://doi.org/10.1016/j.envres.2024.120094
263 Jiang, X., Guo, S., Wang, S., Zhang, Y., Chen, H., Wang, Y., et al. (2022) EIF4A3-Induced circARHGAP29 Promotes Aerobic Glycolysis in Docetaxel-Resistant Prostate Cancer through IGF2BP2/c-Myc/LDHA Signaling. *Cancer Res* **82**, 831–845 https://doi.org/10.1158/0008-5472.CAN-21-2988
264 Watt, M. J., Clark, A. K., Selth, L. A., Haynes, V. R., Lister, N., Rebello, R., et al. (2019) Suppressing fatty acid uptake has therapeutic effects in preclinical models of prostate cancer. *Sci Transl Med* **11**, eaau5758 https://doi.org/10.1126/scitranslmed.aau5758
265 Tessem, M.-B., Bertilsson, H., Angelsen, A., Bathen, T. F., Drabløs, F. and Rye, M. B. (2016) A Balanced Tissue Composition Reveals New Metabolic and Gene Expression Markers in Prostate Cancer. *PLoS One* **11**, e0153727 https://doi.org/10.1371/journal.pone.0153727

266 Lin, S.-R., Wen, Y.-C., Yeh, H.-L., Jiang, K.-C., Chen, W.-H., Mokgautsi, N., et al. (2020) EGFR-upregulated LIFR promotes SUCLG2-dependent castration resistance and neuroendocrine differentiation of prostate cancer. *Oncogene*, Nature Publishing Group **39**, 6757–6775 https://doi.org/10.1038/s41388-020-01468-9
267 Ding, Q., Lu, C., Hao, Q., Zhang, Q., Yang, Y., Olsen, R. E., et al. (2022) Dietary Succinate Impacts the Nutritional Metabolism, Protein Succinylation and Gut Microbiota of Zebrafish. *Front. Nutr.*, Frontiers **9** https://doi.org/10.3389/fnut.2022.894278
268 Nassar, Z. D., Mah, C. Y., Dehairs, J., Burvenich, I. J., Irani, S., Centenera, M. M., et al. (2020) Human DECR1 is an androgen-repressed survival factor that regulates PUFA oxidation to protect prostate tumour cells from ferroptosis. *Elife* **9**, e54166 https://doi.org/10.7554/eLife.54166
269 Blomme, A., Ford, C. A., Mui, E., Patel, R., Ntala, C., Jamieson, L. E., et al. (2020) 2,4-dienoyl-CoA reductase regulates lipid homeostasis in treatment-resistant prostate cancer. *Nat Commun* **11**, 2508 https://doi.org/10.1038/s41467-020-16126-7
270 Chen, J., Wang, Y., Han, L., Wang, R., Gong, C., Yang, G., et al. (2022) A ferroptosis-inducing biomimetic nanocomposite for the treatment of drug-resistant prostate cancer. *Mater Today Bio* **17**, 100484 https://doi.org/10.1016/j.mtbio.2022.100484
271 Bramhecha, Y. M., Guérard, K.-P., Audet-Walsh, É., Rouzbeh, S., Kassem, O., Pernet, E., et al. (2022) Fatty acid oxidation enzyme Δ3, Δ2-enoyl-CoA isomerase 1 (ECI1) drives aggressive tumour phenotype and predicts poor clinical outcome in prostate cancer patients. *Oncogene* **41**, 2798–2810 https://doi.org/10.1038/s41388-022-02276-z
272 Matejcic, M., Patel, Y., Lilyquist, J., Hu, C., Lee, K. Y., Gnanaolivu, R. D., et al. (2020) Pathogenic Variants in Cancer Predisposition Genes and Prostate Cancer Risk in Men of African Ancestry. *JCO Precis Oncol*, Wolters Kluwer 32–43 https://doi.org/10.1200/PO.19.00179
273 Rocca, C., Rocca, G., Zampieri, P., Dell'atti, L., Bianchi, N., Ippolito, C., et al. (2023) Somatic and Germline Variants Affect Prognosis and Susceptibility in Prostate Cancer. *Anticancer Res* **43**, 2941–2949 https://doi.org/10.21873/anticanres.16465
274 Lirussi, L., Antoniali, G., D'Ambrosio, C., Scaloni, A., Nilsen, H. and Tell, G. (2016) APE1 polymorphic variants cause persistent genomic stress and affect cancer cell proliferation. *Oncotarget* **7**, 26293–26306 https://doi.org/10.18632/oncotarget.8477
275 Guo, N., Chen, Y., Zhang, Y., Deng, Y., Zeng, F. and Li, X. (2022) Potential Role of APEX1 During Ferroptosis. *Front Oncol* **12**, 798304 https://doi.org/10.3389/fonc.2022.798304
276 Pieretti, M., Khattar, N. H. and Smith, S. A. (2001) Common polymorphisms and somatic mutations in human base excision repair genes in ovarian and endometrial cancers. *Mutat Res* **432**, 53–59 https://doi.org/10.1016/s1383-5726(00)00002-9
277 Illuzzi, J. L., McNeill, D. R., Bastian, P., Brenerman, B., Wersto, R., Russell, H. R., et al. (2017) Tumour-associated APE1 variant exhibits reduced complementation efficiency but does not promote cancer cell phenotypes. *Environ Mol Mutagen* **58**, 84–98 https://doi.org/10.1002/em.22074
278 Chen, Y., Li, J. and Mo, Z. (2016) Association between the APEX1 Asp148Glu polymorphism and prostate cancer, especially among Asians: a new evidence-based analysis. *Oncotarget* **7**, 52530–52540 https://doi.org/10.18632/oncotarget.9693
279 Gong, Z., Platek, M. E., Till, C., Goodman, P. J., Tangen, C. M., Platz, E. A., et al. (2021) Associations Between Polymorphisms in Genes Related to Oxidative Stress and DNA Repair, Interactions With Serum Antioxidants, and Prostate Cancer Risk: Results From the Prostate Cancer Prevention Trial. *Front Oncol* **11**, 808715 https://doi.org/10.3389/fonc.2021.808715

280 Kuasne, H., Rodrigues, I. S., Losi-Guembarovski, R., Reis, M. B., Fuganti, P. E., Gregório, E. P., et al. (2011) Base excision repair genes XRCC1 and APEX1 and the risk for prostate cancer. *Mol Biol Rep* **38**, 1585–1591 https://doi.org/10.1007/s11033-010-0267-z
281 Juhnke, M., Heumann, A., Chirico, V., Höflmayer, D., Menz, A., Hinsch, A., et al. (2017) Apurinic/apyrimidinic endonuclease 1 (APE1/Ref-1) overexpression is an independent prognostic marker in prostate cancer without TMPRSS2:ERG fusion. *Mol Carcinog* **56**, 2135–2145 https://doi.org/10.1002/mc.22670
282 Chen, C., Zhang, L.-G., Liu, J., Han, H., Chen, N., Yao, A.-L., et al. (2016) Bioinformatics analysis of differentially expressed proteins in prostate cancer based on proteomics data. *Onco Targets Ther* **9**, 1545–1557 https://doi.org/10.2147/OTT.S98807
283 Parente, A. D., Bolland, D. E., Huisinga, K. L. and Provost, J. J. (2024) Physiology of malate dehydrogenase and how dysregulation leads to disease. *Essays Biochem* **68**, 121–134 https://doi.org/10.1042/EBC20230085
284 Vujicic, I., Rusevski, A., Stankov, O., Popov, Z., Dimovski, A. and Davalieva, K. (2022) Potential Role of Seven Proteomics Tissue Biomarkers for Diagnosis and Prognosis of Prostate Cancer in Urine. *Diagnostics*, Multidisciplinary Digital Publishing Institute **12**, 3184 https://doi.org/10.3390/diagnostics12123184
285 Latonen, L., Afyounian, E., Jylhä, A., Nättinen, J., Aapola, U., Annala, M., et al. (2018) Integrative proteomics in prostate cancer uncovers robustness against genomic and transcriptomic aberrations during disease progression. *Nat Commun* **9**, 1176 https://doi.org/10.1038/s41467-018-03573-6
286 Liu, Q., Harvey, C. T., Geng, H., Xue, C., Chen, V., Beer, T. M., et al. (2013) Malate dehydrogenase 2 confers docetaxel resistance via regulations of JNK signaling and oxidative metabolism. *Prostate* **73**, 1028–1037 https://doi.org/10.1002/pros.22650
287 Li, C., He, C., Xu, Y., Xu, H., Tang, Y., Chavan, H., et al. (2019) Alternol eliminates excessive ATP production by disturbing Krebs cycle in prostate cancer. *Prostate* **79**, 628–639 https://doi.org/10.1002/pros.23767
288 Chen, B., Liu, Y., He, Y. and Shen, C. (2024) Pan-cancer analysis of prognostic and immunological role of IL4I1 in human tumours: a bulk omics research and single cell sequencing validation. *Discov Onc* **15**, 139 https://doi.org/10.1007/s12672-024-01000-5
289 Rao, D., Yu, C., Wang, T., Sheng, J., Lv, E., Liang, H., et al. (2022) Pan-cancer analysis combined with experimental validation revealed IL4I1 as an immunological and prognostic biomarker. *International Immunopharmacology* **111**, 109091 https://doi.org/10.1016/j.intimp.2022.109091
290 Cm, S., C, C., P, P., M, A. and D, V. (2015) Differential expression of the immunosuppressive enzyme IL4I1 in human induced Aiolos+, but not natural Helios+, FOXP3+ Treg cells. *European journal of immunology*, Eur J Immunol **45** https://doi.org/10.1002/eji.201444897
291 Run, L., Tian, Z., Xu, L., Du, J., Li, N., Wang, Q., et al. (2023) Knockdown of IL4I1 Improved High Glucose-evoked Insulin Resistance in HepG2 Cells by Alleviating Inflammation and Lipotoxicity Through AHR Activation. *Appl Biochem Biotechnol* **195**, 6694–6707 https://doi.org/10.1007/s12010-023-04399-9
292 Stamis, S. A., Heath, E. I., Lucas, S., Boerner, J. and Slusher, L. B. (2022) Alcohol dehydrogenase expression patterns in normal prostate, benign prostatic hyperplasia, and prostatic adenocarcinoma in African American and Caucasian men. *The Prostate* **82**, 666–675 https://doi.org/10.1002/pros.24310
293 Dairo, O., DePaula Oliveira, L., Schaffer, E., Vidotto, T., Mendes, A. A., Lu, J., et al. (2024) FASN Gene Methylation is Associated with Fatty Acid Synthase Expression and

Clinical-genomic Features of Prostate Cancer. *Cancer Res Commun* **4**, 152–163 https://doi.org/10.1158/2767-9764.CRC-23-0248

294 Chianese, U., Papulino, C., Ali, A., Ciardiello, F., Cappabianca, S., Altucci, L., et al. (2023) FASN multi-omic characterization reveals metabolic heterogeneity in pancreatic and prostate adenocarcinoma. *J Transl Med* **21**, 32 https://doi.org/10.1186/s12967-023-03874-5

295 Stoykova, G. E. and Schlaepfer, I. R. (2019) Lipid Metabolism and Endocrine Resistance in Prostate Cancer, and New Opportunities for Therapy. *Int J Mol Sci* **20**, 2626 https://doi.org/10.3390/ijms20112626

296 Liu, M., Pan, Q., Xiao, R., Yu, Y., Lu, W. and Wang, L. (2020) A cluster of metabolism-related genes predict prognosis and progression of clear cell renal cell carcinoma. *Sci Rep*, Nature Publishing Group **10**, 12949 https://doi.org/10.1038/s41598-020-67760-6

297 Mattu, S., Fornari, F., Quagliata, L., Perra, A., Angioni, M. M., Petrelli, A., et al. (2016) The metabolic gene HAO2 is downregulated in hepatocellular carcinoma and predicts metastasis and poor survival. *J Hepatol* **64**, 891–898 https://doi.org/10.1016/j.jhep.2015.11.029

298 Wang, H., Liu, Z., Wang, Y., Han, D., Du, Y., Zhang, B., et al. (2023) Comprehensive analysis of fatty acid metabolism-related gene signatures for predicting prognosis in patients with prostate cancer. *PeerJ*, PeerJ Inc. **11**, e14646 https://doi.org/10.7717/peerj.14646

299 Xing, C., Ci, X., Sun, X., Fu, X., Zhang, Z., Dong, E. N., et al. (2014) *Klf5* Deletion Promotes *Pten* Deletion–Initiated Luminal-Type Mouse Prostate Tumours through Multiple Oncogenic Signaling Pathways. *Neoplasia* **16**, 883–899 https://doi.org/10.1016/j.neo.2014.09.006

300 Tseng, C.-C., Hung, C.-C., Shu, C.-W., Lee, C.-H., Chen, C.-F., Kuo, M.-S., et al. (2023) The Clinical and Biological Effects of Receptor Expression-Enhancing Protein 6 in Tongue Squamous Cell Carcinoma. *Biomedicines*, Multidisciplinary Digital Publishing Institute **11**, 1270 https://doi.org/10.3390/biomedicines11051270

301 Gupta, N., Ochiai, H., Hoshino, Y., Klein, S., Zustin, J., Ramjiawan, R. R., et al. (2023) Inhibition of CXCR4 Enhances the Efficacy of Radiotherapy in Metastatic Prostate Cancer Models. *Cancers (Basel)* **15**, 1021 https://doi.org/10.3390/cancers15041021

302 Xia, L., Wang, H., Du, G., Cheng, X., Zhang, R., Yu, H., et al. (2024) Receptor accessory protein 6, a novel ferroptosis suppressor, drives oral squamous cell carcinoma by maintaining endoplasmic reticulum hemostasis. *International Journal of Biological Macromolecules* **283**, 137565 https://doi.org/10.1016/j.ijbiomac.2024.137565

303 Frottin, F., Bienvenut, W. V., Bignon, J., Jacquet, E., Vaca Jacome, A. S., Van Dorsselaer, A., et al. (2016) MetAP1 and MetAP2 drive cell selectivity for a potent anti-cancer agent in synergy, by controlling glutathione redox state. *Oncotarget* **7**, 63306–63323 https://doi.org/10.18632/oncotarget.11216

304 Ehlers, T., Furness, S., Robinson, T. P., Zhong, H. A., Goldsmith, D., Aribser, J., et al. (2016) Methionine AminoPeptidase Type-2 Inhibitors Targeting Angiogenesis. *Current Topics in Medicinal Chemistry*, Bentham Science Publishers **16**, 1478–1488 https://doi.org/10.2174/1568026615666150915121204

305 Yılmaz, Ö., Bayer, B., Bekçi, H., Uba, A. I., Cumaoğlu, A., Yelekçi, K., et al. (2020) Synthesis, Anticancer Activity on Prostate Cancer Cell Lines and Molecular Modeling Studies of Flurbiprofen-Thioether Derivatives as Potential Target of MetAP (Type II). *Medicinal Chemistry* **16**, 735–749 https://doi.org/10.2174/1573406415666190613162322

306 Ribeiro, E. and Vale, N. (2023) Repurposing of the Drug Tezosentan for Cancer Therapy. *Current Issues in Molecular Biology*, Multidisciplinary Digital Publishing Institute **45**, 5118–5131 https://doi.org/10.3390/cimb45060325
307 Williams, D. E. (2021) Indoles Derived From Glucobrassicin: Cancer Chemoprevention by Indole-3-Carbinol and 3,3'-Diindolylmethane. *Front. Nutr.*, Frontiers **8** https://doi.org/10.3389/fnut.2021.734334
308 Novick, R. M., Vezina, C. M. and Elfarra, A. A. (2010) Isoform distinct time-, dose-, and castration-dependent alterations in flavin-containing monooxygenase expression in mouse liver after 2,3,7,8-tetrachlorodibenzo-*p*-dioxin treatment. *Biochemical Pharmacology* **79**, 1345–1351 https://doi.org/10.1016/j.bcp.2009.12.020